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Attorney Docket No. 8616.CIP3.CON

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith for filing under 37 C.F.R. § 1.53(b) is a patent application comprising:

Sixty-two (62) pages of specification, claims and abstract.

Thirteen (13) sheets of X formal/____ informal drawings.

X Declaration, Power of Attorney and Petition.

____ Newly Executed

X Copy from prior application (when a continuation or divisional)(37 C.F.R.§ 1.63(d))

CERTIFICATE OF MAILING BY EXPRESS MAIL

Mailed: August 11th, 1998

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service on the date indicated above in an envelope addressed to: Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

Respectfully submitted,

Grant R. Clayton
Attorney for Applicant

__X__ Incorporation by Reference (if a copy of the declaration from prior application is included). The entire disclosure of the prior application, from which a copy of the declaration is supplied herewith as referenced above, is considered as being part of the disclosure of the accompanying application ans is hereby incorporated by reference therein.

_____ Microfiche Computer Program (Appendix)

_____ Nucleotide and/or Amino Acid Sequence Submission

_____ Computer Readable Copy

_____ Paper Copy (identical to computer readable copy)

_____ Statement verifying identity of above referenced copies

__X__ This application is a Continuing application and a

__X__ Continuation of prior application no. 08/537,612

_____ Divisional of prior application no. ___

_____ Continuation-in-part of prior application no. _____

Also enclosed are:

_____ An Assignment from _____ to _____ (including assignment cover sheet)

_____ Information Disclosure Statement (IDS) including PTO-1449_____ Copies of _____ (_____ _____) references

__X__ Preliminary Amendment

__X__ Return Receipt Postcard (MPEP 503) Should be specially itemized

_____ Declaration Claiming Small Entity Status for _____

__X__ A Declaration Claiming Small Entity Status was filed in the prior application and such status is still proper and desired

_____ Certified copy of priority document(s) (if foreign priority is claimed)

_____ A copy of the extension of time simultaneously filed in the prior application

FEES

_____ No fees are enclosed.

__X__ Check No. 4257 in the amount of $636.00 which represents the filing fee for this application.

The fees have been calculated as set forth below.

PATENT APPLICATION FEE CALCULATION TABLE							
			SMALL ENTITY			LARGE ENTITY	
	NO. FILED	NO. EXTRA	RATE	FEE	OR	RATE	FEE
BASIC FEE				$ 395	OR		$ 0
TOTAL CLAIMS	27 - 20 =	7	X 11 =	$ 77	OR	X 22 =	$
IND. CLAIMS	7 - 03 =	4	X 41 =	$ 164	OR	X 82 =	$
Presentation of Multiple Dependent Claim			135 =	$ 0	OR	270 =	
ASSIGNMENT FILING FEE (___)			X 40 =	$ 0	OR	X 40 =	$
			TOTAL:	$ 636	OR	TOTAL:	$ 0

The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or to credit any overpayment to Deposit Account No. 20-0100. An original and one copy of this letter are enclosed.

__X__ Any and all filing fees required under 37 C.F.R. § 1.16.

__X__ Any patent application processing fees under 37 C.F.R. § 1.17.

The Commissioner is hereby authorized to charge payment of the following fees during the pendency of this application or credit any overpayment to Deposit Account No. 20-0100.

__X__ Any patent application processing fees under 37 C.F.R. § 1.17.

_____ The issue fee set in 37 C.F.R. § 1.18 at or before mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

__X__ Any filing fees under 37 C.F.R. § 1.16 for presentation of extra claims.

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__X__ Please refer to the following attorney docket number in all future correspondence and telecommunications:

Attorney Docket No. 8616.CIP3.CON

DATED this 11th day of August, 1998.

Respectfully submitted,

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EXPRESS MAIL LABEL NO. EL108261870US

PATENT APPLICATION
Docket No. 8616.CIP3CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	Carl T. Wittwer, et al.	
Serial No.:	_____	Art Unit _____
Filed:	August 11, 1998	
Continuation Application of:		
Serial No.:	08/537,612	Art Unit 1743
Filed:	October 2, 1995	
For:	METHOD FOR RAPID THERMAL CYCLING OF BIOLOGICAL SAMPLES	
Examiner:	J. M. Ludlow	

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents
and Trademarks
Washington, D. C. 20231

Sir:

Prior to calculation of the filing fee and examination of this application, please enter this amendment.

IN THE SPECIFICATION:

Please amend the Specification at page 2, lines 5-8 as follows:

--This application is a continuation of U.S. patent application serial no. 08/537,612 filed October 2, 1995 entitled Method For Rapid Thermal Cycling of Biological Samples which is a continuation-in-part of U.S. patent application serial no. 08/179,969 filed January 10, 1994 entitled Rapid Thermal Cycling Device, now U.S. Patent No. 5,455,175 issued on October 3, 1995[.] which is a continuation-in-part of U.S. patent application serial no. 07/815,966 filed January 2, 1992 entitled Rapid Thermal Cycling Device, now abandoned, which is a continuation-in-part of U.S. patent application serial no. 07/534,029 filed June 4, 1990 entitled Automated Polymerase Chain Reaction Device, now abandoned.--

IN THE CLAIMS:

Please cancel claims 1-29, without prejudice.

Please add new claims 30-56, as indicated below.

30. An improved method of amplifying a nucleic acid sequence by thermal cycling of the nucleic acid sequence in the presence of a thermostable DNA polymerase, said method comprising the steps of:

--This application is a continuation of U.S. patent application serial no. 08/537,612 filed October 2, 1995 entitled Method For Rapid Thermal Cycling of Biological Samples which is a continuation-in-part of U.S. patent application serial no. 08/179,969 filed January 10, 1994 entitled Rapid Thermal Cycling Device, now U.S. Patent No. 5,455,175 issued on October 3, 1995[.] which is a continuation-in-part of U.S. patent application serial no. 07/815,966 filed January 2, 1992 entitled Rapid Thermal Cycling Device, now abandoned, which is a continuation-in-part of U.S. patent application serial no. 07/534,029 filed June 4, 1990 entitled Automated Polymerase Chain Reaction Device, now abandoned.--

IN THE CLAIMS:

Please cancel claims 1-29, without prejudice.

Please add new claims 30-56, as indicated below.

30. An improved method of amplifying a nucleic acid sequence by thermal cycling of the nucleic acid sequence in the presence of a thermostable DNA polymerase, said method comprising the steps of:

(a) placing a biological sample comprising said nucleic acid sequence in a capillary vessel;

(b) raising the temperature of the biological sample from a first temperature to a second temperature wherein the second temperature is at least 15°C higher than the first temperature;

(c) holding the biological sample at the second temperature for a predetermined amount of time;

(d) lowering the temperature of the biological sample from the second temperature to at least the first temperature;

(e) holding the biological sample at a temperature at least as low as the first temperature for a pre-determined length of time;

(f) raising the temperature of the biological sample to the second temperature; and

(g) repeating steps c through f, wherein those steps are completed in approximately 60 seconds or less.

31. The improved method of claim 30 wherein the capillary vessel defines a volume ranging from about 10 microliters to about 100 microliters.

32. The improved method of claim 30 wherein the pre-determined length of time for holding step (c) is less than one second.

33. The improved method of claim 30 wherein step (d) comprises lowering the temperature of the biological sample to a third temperature that is below the first temperature, step (e) comprises holding the biological sample at the third temperature for a pre-determined length of time, said method further comprising the step of raising the temperature of the biological sample back to the first temperature and holding the sample at the first temperature for a pre-determined length of time before proceeding to step (f).

34. The improved method of claim 33 wherein the pre-determined length of time for holding step (e) is less than one second.

35. An improved method of modifying a biological sample by subjecting the biological sample to multiple cycles of controlled rapid heating and cooling, said method comprising the steps of placing the sample in a capillary vessel and thermally cycling the

sample by contacting the capillary vessel with a heated fluid to raise the temperature of the sample from a first temperature to a second temperature, cooling the sample from said second temperature to a temperature at least as low as the first temperature, and heating the sample back to the second temperature; wherein the difference between the first temperature and the second temperature is at least 15°C, and wherein the heating and cooling step are completed in approximately 60 seconds or less, while the temperature homogeneity in the sample is maintained within plus or minus 1°C during the heating and cooling steps.

36. In a method of amplifying a DNA sequence by thermal cycling of the nucleic acid sequence in the presence of a thermostable DNA polymerase, wherein each cycle comprises the steps of heating a biological sample containing said DNA sequence to a denaturing temperature; holding the biological sample at the denaturing temperature; cooling the sample to an annealing temperature, holding the temperature at the annealing temperature for a pre-determined amount of time; warming the sample to an elongation temperature; and holding the sample at the elongation temperature for a predetermined amount of time; the improvement

comprising limiting the holding time of the annealing and denaturing steps to less than one second each.

37. A method for improving the purity of a product produced by polymerase chain reaction wherein a DNA sequence is amplified by thermal cycling of the sequence in an aqueous sample in the presence of a thermostable polymerase and wherein each thermal cycle comprises heating the aqueous sample to a denaturation temperature, holding it at the denaturation temperature for a predetermined period of time, cooling the sample to an annealing temperature and holding it at the annealing temperature for a predetermined period of time, the method comprising the step of limiting the time the sample is held at the annealing temperature to less than 5 seconds.

38. The method of claim 37 wherein the sample is held at the annealing temperature for less than one second.

39. The method of claim 37 further comprising the step of cooling the sample from the denaturation temperature to the annealing temperature at a rate of at least about 1.48°C per second.

40. The method of claim 37 wherein each thermal cycle is completed in less than 60 seconds.

41. The method of claim 37 wherein the period of time the sample is held at the denaturation temperature during each thermal cycle is less than 32 seconds.

42. The method of claim 41 wherein the period of time the sample is held at the denaturation temperature during each thermal cycle is less than one second.

43. A method for increasing the yield of an amplified DNA sequence by use of polymerase chain reaction wherein the DNA sequence is thermally cycled in an aqueous sample in the presence of a thermostable polymerase and wherein during each thermal cycle the sample is heated to a denaturation temperature and held for a predetermined period of time, said method comprising the step of limiting the time the sample is held at the denaturation temperature to less than 32 seconds.

44. The method of claim 43 wherein the sample is held at the denaturation temperature for less than one second.

45. The method of claim 43 wherein each thermal cycle is completed in less than approximately 60 seconds.

46. In the construction of a thermal cycling device suitable for performing polymerase chain reaction DNA amplification in which the device comprises a closed-loop system, a fan for moving air in the closed-loop system to transfer heat from a heat source to a sample compartment in said closed-loop system, a temperature sensor in the sample compartment, and a programmable temperature controller for controlling the temperature in the sample compartment, the improvement which comprises minimizing the thermal mass of the device components in the closed-loop system in contact with the air, installing air mixing baffles in the closed-loop system, and selecting the fan and the heat source so that the temperature in the sample compartment can be cycled between a DNA denaturation temperature of at least 90° and a DNA-annealing temperature in the range of about 50° to about 55°C in approximately 30 to approximately 60 seconds while maintaining a temperature gradient in the sample compartment of less than 2°C.

47. The improvement of claim 46 wherein the fan has the capacity of at least 75 cubic feet of air per minute.

48. A thermal cycling device for optimizing yield and purity of amplified DNA prepared by a process comprising thermal cycling of an aqueous sample comprising a DNA sequence and a thermostable polymerase between a DNA denaturation temperature and a lower DNA annealing temperature, said device comprising

a chamber for substantially confining and directing flow of air contained therein along a closed-loop path,

a sample compartment located in said chamber within said closed-loop path, a fan for moving air along said closed-loop path, means for heating air in said closed-loop path,

means for mixing air in the closed-loop path between the air heating means and the sample compartment,

a thermal sensor for providing a signal related to the temperature of said sample compartment,

a vent in the chamber to direct air from the closed-loop path and means for opening and closing said vent, and

a programmable controller for receiving the signal from the thermal sensor and for controlling the air-heating means and the vent opening and closing means to cycle the temperature of air in the sample compartment wherein the controller is programmable to cycle the temperature in the sample compartment between a DNA-denaturation temperature and a DNA-annealing temperature in 60

seconds or less while maintaining a temperature gradient in the sample compartment of less than 10°C.

49. The device of claim 48 wherein during thermal cycling, the temperature gradient in the sample compartment is less than 2°C.

50. The device of claim 48 wherein during thermal cycling the temperature of a sample in the sample compartment can be held at a DNA-denaturation temperature or a DNA-annealing temperature for less than 1 second.

51. The device of claim 48 wherein the temperature in the sample chamber can be cycled between a DNA-denaturation temperature and a DNA-annealing temperature in approximately 30 seconds.

52. The device of claim 51 wherein during thermal cycling, the temperature gradient in the sample compartment is less than 2°C.

53. The device of claim 48 constructed so that the thermal mass of the device components in contact with air in the closed-loop path is minimized.

54. The device of claim 48 wherein in the capacity of the fan is at least 75 ft.3/min.

55. The device of claim 48 wherein the temperature of a sample in the sample compartment can be uniformly cycled at a rate of temperature change of at least 1.48°C/sec.

56. The device of claim 48 wherein the temperature of a sample in the sample compartment can he uniformly cycled at a rate of temperature change of at least 4.11°C/sec.

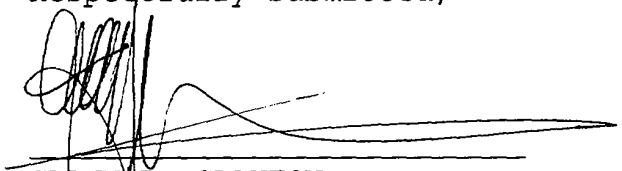
REMARKS

After entry of this amendment, it is believed that all of the claims now pending are allowable. Thus, favorable action concerning these claims is respectfully requested. If any impediment to the allowance of these claims remains after entry of this Amendment and consideration of these remarks the Examiner is invited to initiate a telephone interview with the undersigned.

Any deficiency in any fee submitted in connection with this paper may be charged to Deposit Account 20-0100.

DATED this 11th day of August, 1998.

Respectfully submitted,



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EXPRESS MAIL LABEL NO. EL108261870US

PATENT APPLICATION
Attorney Docket
No. 8616.CIP3.CON

METHOD FOR RAPID THERMAL CYCLING OF BIOLOGICAL SAMPLES

BACKGROUND

1. Related Applications.

This application is a continuation-in-part of U.S. patent application serial no. 08/179,969 filed January 10, 1994 entitled Rapid Thermal Cycling Device, U.S. Patent No. 5,455,175 issued on October 3, 1995.

2. The Field of the Invention.

This invention relates generally to apparatus for rapid control of the temperature of a liquid sample. More specifically, the present invention relates to thermal cycling apparatus for precisely controlling and rapidly varying the temperature of a sample repeatedly through a predetermined temperatures cycle.

3. The Background Art.

In numerous areas of industry, technology, and research there is a need to reliably and reproducibly subject relatively small samples to thermal cycling. The need to

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subject a sample to repeated thermal cycles is particularly acute in biotechnology applications. In the biotechnology field, it is often desirable to repeatedly heat and cool small samples of materials over a short period of time. One such biological process that is regularly carried out is cyclic DNA amplification.

Cyclic DNA amplification, using a thermostable DNA polymerase, allows automated amplification of primer specific DNA, widely known as the "polymerase chain reaction." Automation of this process requires controlled and precise thermal cycling of reaction mixtures usually contained in a plurality of containers. In the past, the container of preference has been a standard, plastic microfuge tube.

Commercial programmable metal heat blocks have been used in the past to effect the temperature cycling of samples in microfuge tubes through the desired temperature versus time profile. However, the inability to quickly and accurately adjust the temperature of the heat blocks through a large temperature range over a short time period, has rendered the use of heat block type devices undesirable as a heat control system when carrying out the polymerase chain reaction.

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Moreover, the microfuge tubes which are generally used have disadvantages. The material of the microfuge tubes, their wall thickness, and the geometry of microfuge tubes is a hinderance to rapid heating and cooling of the sample contained therein. The plastic material and the thickness of the wall of microfuge tubes act as an insulator between the sample contained therein and the surrounding medium thus hindering transfer of thermal energy. Also, the geometry of the microfuge tube presents a small surface area to whatever medium is being used to transfer thermal energy. The continued use of microfuge tubes in the art, with their suboptimal geometry, indicates that the benefits of improved thermal transfer (which come by increasing the surface area of a sample container for a sample of constant volume) has heretofore not been recognized.

Furthermore, devices using water baths with fluidic switching, (or mechanical transfer) have also been used as a thermal cycler for the polymerase chain reaction. Although water baths have been used in cycling a polymerase chain reaction mixture through a desired temperature versus time profile necessary for the reaction to take place, the high

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thermal mass of the water (and the low thermal conductivity of plastic microfuge tubes), has been significantly limiting as far as performance of the apparatus and the yields of the reaction are concerned.

Devices using water baths are limited in their performance. This is because the water's thermal mass significantly restricts the maximum temperature versus time gradient which can be achieved thereby. Also, the water bath apparatus has been found to be very cumbersome due to the size and number of water carrying hoses and external temperature controlling devices for the water. Further the need for excessive periodic maintenance and inspection of the water fittings for the purpose of detecting leaks in a water bath apparatus is tedious and time consuming. Finally, it is difficult with the water bath apparatus to control the temperature in the sample tubes with the desired accuracy.

U.S. Patent No. 3,616,264 to Ray shows a thermal forced air apparatus for cycling air to heat or cool biological samples to a constant temperature. Although the Ray device is somewhat effective in maintaining a constant temperature within an air chamber, it does not address the need for

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rapidly adjusting the temperature in a cyclical manner according to a temperature versus time profile such as is required for biological procedures such as the polymerase chain reaction.

U.S. Patent No. 4,420,679 to Howe and U.S. Patent No. 4,286,456 to Sisti et al. both disclose gas chromatographic ovens. The devices disclosed in the Howe and Sisti et al. patents are suited for carrying out gas chromatography procedures but do not provide thermal cycling which is substantially any more rapid than that provided by any of the earlier described devices. Rapid thermal cycling, while potentially useful for many procedures, is particularly advantageous for carrying out the polymerase chain reaction. Devices such as those described in the Howe and Sisti et al. patents are not suitable for efficiently and rapidly carrying out such reactions.

BRIEF SUMMARY AND OBJECTS OF THE INVENTION

In view of the above described state of the art, the present invention seeks to realize the following objects and advantages.

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It is an object of the present invention to provide an apparatus for accurately controlling the temperature of biological samples.

It is a further object of the present invention to provide a thermal cycling apparatus for quickly and accurately varying the temperature of biological samples according to a predetermined temperature versus time profile.

It is another object of the present invention to provide an apparatus suitable for subjecting a number of different biological samples to rapid thermal cycling.

It is also an object of the present invention to provide a thermal cycling apparatus having a thermal transfer medium of low thermal mass which can effectively subject samples to a large temperature gradient over a very short period of time.

It is a further object of the present invention to provide an apparatus which can subject a biological sample to rapid thermal cycling using air as a thermal transfer medium.

It is another object of the present invention to provide a thermal cycling apparatus which will heat samples located in a fluid chamber therein, by means of an internal heater, and will subsequently cool the samples by moving ambient fluid

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into the chamber, at the proper time in the thermal cycle, to cool the samples.

These and other objects and advantages of the invention will become more fully apparent from the description and claims which follow, or may be learned by the practice of the invention.

The present invention is an apparatus particularly suited for subjecting biological samples to rapid thermal cycling in order to carry out one or more of a number of procedures or processes. In one of its preferred forms, the apparatus includes a means for holding a biological sample. The means for holding a biological sample, or a sample chamber, is provided with an insulation means for retaining thermal energy and also a means for heating the interior of the sample chamber. Preferably, a high wattage incandescent lamp functions as a means for heating the interior of the sample chamber. A thermal insulator lines the interior of the sample chamber and functions to retain the heat generated by the lamp within the sample chamber and serves as an insulation means.

In order to rapidly cool the sample chamber, the preferred apparatus includes a means for forcing air into the

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sample chamber and a means for dispersing the air forced into the sample chamber. A high velocity fan functions to force air into the sample chamber and a rotating paddle functions to disperse the air into the chamber. A means for venting allows the air to escape from the sample chamber taking the unwanted heat with it. The present invention allows heating and cooling of a sample to take place both quickly and uniformly.

The apparatus of the present invention includes a control means for operating the apparatus through the desired time versus temperature profile. The present invention is particularly well suited for carrying out automated polymerase chain reactions.

Other embodiments of the present invention include a closed loop hot fluid compartment and a reaction compartment. The reaction compartment is located within the hot fluid compartment and can be accessed through a venting door for allowing the insertion of samples in capillary tubes which may contain a reaction mixture for the polymerase chain reaction. A heating coil is also located in the compartment and is regulated by a programmable set-point process controller via

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a thermocouple sensor which is also located in the compartment at a position directly adjacent the reaction compartment.

The heat coil is located up-stream of the reaction compartment, and a fan is located up-stream of the heat coil, such that, fluid blown across the heat coil by the blower unit passes through the reaction compartment and into the intake side of the blower unit in closed loop fashion. Baffles may be located between the heat coil and the reaction compartment in order to cause uniform homogenous mixing of the heated fluid before it passes through the reaction compartment.

Alternately, the fan may be placed downstream of the heating coil but before the reaction compartment. In this case, the fan blades serve as baffles to mix the heated fluid. At the correct time in the predetermined thermal cycle, the controller activates a solenoid that opens the venting door which vents fluid out of the compartment and allows cool (ambient) fluid to enter and cool the samples.

The controller of the present invention allows the chamber, and subsequently the samples located in the sample compartment therein, to pass through a predetermined temperature cycle corresponding to the denaturation, annealing

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and elongation steps in the polymerase chain reaction. In use, the apparatus of the present invention allows rapid optimization of denaturation, annealing, and elongation steps in terms of time and temperature, and shortened time periods (ramp times) between the temperatures at each step.

The present invention particularly decreases the total time required for completion of polymerase chain reaction cycling over prior art thermal cycling devices while at the same time significantly increasing specificity and yield.

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BRIEF DESCRIPTION OF THE DRAWINGS

In order to better appreciate how the above-recited and other advantages and objects of the invention are obtained, a more particular description of the invention briefly described above will be rendered by reference to specific embodiments thereof which are illustrated in the appended drawings. Understanding that these drawings depict only a typical embodiment of the invention and are not therefore to be considered limiting of its scope, the invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

Figure 1 shows a perspective view of a thermal cycling apparatus adapted for thermal cycling of biological samples and adapted especially for use in cyclic DNA amplification, according to the concepts of the present invention;

Figure 2 is a side elevation view of the fluid chamber portion of the apparatus of Figure 1;

Figure 3 is an interior plan view of the fluid chamber portion of the apparatus illustrated in Figure 1;

Figure 4 shows an interior plan view of the fluid chamber of another embodiment of the present invention;

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Figure 5 shows an optimized temperature versus time profile for a polymerase chain reaction using the thermal cycling device of the present invention;

Figure 6 shows graphically the effect of annealing time on polymerase chain reaction specificity and yields using the thermal cycling device of the present invention;

Figure 7 shows graphically the effect of denaturation time on polymerase chain reaction yields using the thermal cycling device of the present invention;

Figures 8A-B, which are perspective and elevational cross sectioned views, respectively, of another preferred embodiment of the present invention;

Figure 8C is a diagrammatic representation of the relationship of the heat producing element and the capillary tubes holding the biological samples in the embodiment illustrated in Figures 8A-B;

Figure 9A shows the results of four different temperature/time profiles (A-D) and their resultant amplification products after thirty cycles (A-D).

Figure 9B shows a cycle of another preferred temperature/time profile used by the present invention;

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Figures 9C-G show exemplary cycle of other preferred temperature/time profiles used by the present invention; and

Figure 10 provides a block diagram of a temperature slope control circuit in accordance with the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference will now be made to the drawings wherein like structures will be provided with like reference designations.

As shown in Figure 1, the thermal cycling device 10 includes a closed loop fluid (most preferably air) chamber, generally designated at 11, which is adapted to accept samples to be cycled through vent door 14. The closed loop fluid chamber 11 includes a plurality of compartments each of which will be described shortly. The device 10 also includes a controller 12 which can be programmed by means of input keys 25 and display 26 to cause the chamber 11 to be cycled through a series of temperatures over a predetermined period of time. The thermal cycling of chamber 11 can be used to carry out numerous procedures and is particularly suited for amplification of primer specific DNA from samples containing reaction mixture as will be explained below.

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The closed loop fluid chamber 11 is enclosed in a generally box shaped configuration by housing 13. Blower mounting boards 16, if desired, can be located so as to section off a smaller rectangular section of the chamber 11 and function to support and secure a generally cylindrically shaped lower housing 15 thereto. Alternatively, the fan of the blower 28 may be housed integrally within chamber housing 13.

The interior of blower housing 15 contains the blades and shaft of the blower. The blower motor (not shown) is located externally of blower housing 15, and therefore exteriorly of the enclosed chamber 11. In this configuration, the blades and shaft are the only parts of the blower which become exposed to the circulating hot fluid within chamber 11. It would be disadvantageous to mount the motor within the chamber which would subject the motor to temperature variations and also would add the thermal mass of the motor to that which is subject to heating and cooling. The reduction of thermal mass exposed to the fluid in chamber 11 is important to the overall performance of the device 10 in its function of subjecting

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samples placed therein to predetermined temperature versus time profiles as will be more fully explained below.

The blower 28 is a well known type blower usually identified as an "in line" type blower which preferably employs a propeller type fan due to its generally low thermal mass, or if desired, a squirrel cage type fan, the fan preferably having a 75 cubic feet per minute minimum capacity.

The solenoid platform 17 has secured thereto a solenoid 18. The solenoid armature 19 is attached to upper end 21 of rod 20 which is rigidly attached to vent door 14 and rotatably attached to housing 13 at points above and below the vent door 14. The rod 20 therefore allows vent door 14 to freely rotate relative to the housing 13 about the rod's longitudinal axis.

A spring 22 is attached at one of its ends to the housing 13 by support post 23. The opposite end of spring 22 is attached to the top end 21 of rod 20 directly adjacent the attachment of solenoid armature 19. The spring 22 is drawn between these two attachment points so as to be in tension. The spring 22 therefore tends to draw top end 21 toward the support post 23, which in turn tends to rotate vent door 14 to its closed position. When solenoid 18 is actuated, armature

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19 tends to pull top end 21 of the rod 20 in the direction of the solenoid 18, which is opposite the direction of pull of spring 22, and which tends to open the vent door 14.

Controller, generally designated at 12, is electrically attached to the chamber 11 by means of a transmission cable 24. The cable 24 also supplies power to the blower motor (not shown), and to the heat coil 31. Further, the controller 12 also is connected to thermocouple sensor 35 for receiving signals corresponding to temperature data, and to solenoid 18 for triggering the solenoid armature.

Controller 12 can be any well known type of temperature controller unit which is programmable to control the heat coil 31, vent door 14, and blower so as to achieve predetermined temperatures as a function of time within the chamber 11, and which is also capable of being programmed to actuate a relay output for driving a solenoid at predetermined time periods and chamber temperature levels. A preferred temperature controller 12 for use in the embodiment of Figures 1-3 is a Partlow MIC-6000 proportional temperature controller, available through Omega Engineering Inc, of Stanford, Connecticut, as the Model No. CN8600 process controller.

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As shown in Figures 2 and 3, the interior of chamber 11 is sectioned off into four main compartments. The blower compartment 28 is formed of the blower housing 15 and the blower mounting plates 16. The entirety of blower compartment 28 is filled with the fan and shaft portions of a blower as has been described above. The blower can be any of a number of well-known designs, as has been described above, and has therefore been omitted from Figure 3 for purposes of clarity. It is sufficient for the present invention to understand that the fan located in blower compartment 28 draws fluid into the blower compartment 28 through inlet opening 36 and pushes the fluid out of exit opening 37.

It is preferred that the fluid be driven by the blower at a rate of at least 75 cubic feet per minute. It is important however, in regard to the present invention, to realize that the fluid located in chamber 11 only contacts the fan and a portion of the drive shaft of the blower, the blower motor itself being located outside of the blower housing 15 so as to avoid any contact thereof with fluid in the chamber 11. This is important since it is critical to the speed of operation of the invention to minimize the material which contacts the

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fluid inside the chamber 11 so as to minimize the thermal mass of material which must be heated and/or cooled thereby during the cycling process. By minimizing the thermal mass which must be heated or cooled by the fluid, the response time necessary to bring the contents of chamber 11 to a uniform temperature is greatly diminished.

Fluid exiting blower compartment 28 through outlet opening 37 enters heating compartment 29. Fluid passing into heating compartment 29 must pass by heating coils 31. If heating coils 31 gets hotter than the fluid passing into heating compartment 29, the fluid will become heated thereby as it is forced through the compartment. The heating coil is preferably a 1,000 watt (125 VAC) nichrome wire coil wound around a microsupport. However, any heating unit suitable for heating the type of fluid present in the chamber may be used. The particular heating coil of embodiment of Figures 1-3 is manufactured by Johnstone Supply, of Portland, Oregon.

The heating coil is activated by an output relay included in the controller 12. The preferred relay is a 25 A, 125 VAC solid state relay manufactured by Omega Engineering Inc. of Stanford, Connecticut as Model No. Omega SSR 240 D25.

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Fluid passing through heating compartment 29 becomes incident on baffles 32 and 33 before passing into the reaction compartment 30. Baffles 32 and 33 tend to break up any laminar fluid flow and generate turbulence therein to effectively mix the fluid so that it arrives in reaction compartment 30 at an homogenous temperature.

Thermocouple sensor 35 provides an electrical input signal to controller 12 which corresponds to the fluid temperature in the reaction compartment 30. Temperature monitoring during operation of the thermal cycling device 10 is preferably achieved by a 30-gauge iron-constantan "J-type" thermocouple. The controller uses this information to regulate the heat coil 31 according to the predetermined temperature versus time profiles programmed therein and to actuate solenoid 18, as will be explained momentarily.

The fluid passing from the reaction compartment 30 to the return air compartment 34 must pass through sample compartment 27 (as shown in dashed lines). Sample compartment 27 will also be explained momentarily.

The fluid in return compartment 34 has been slightly cooled due to the heat transfer therefrom into samples located

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in sample compartment 27. The fluid in return compartment 34 is drawn through inlet opening 36 into blower compartment 28 where it is again forced, by action of the fan, out through outlet opening 37 into the heating compartment 39. Thus, the fluid chamber 11, when operating with vent door 14 closed, is a closed loop fluid chamber which continuously recirculates the fluid along a closed loop path through each compartment thereof in order to bring the contents therein to a uniform temperature. Continuous circulation of the air in the air chamber 11 allows the samples in sample compartment 27 to be brought to a predetermined temperature as quickly as possible, and then to be held at that temperature, if desired.

When the device 10 must be used to not only heat material located in the reaction compartment 27, but also to subsequently cool these materials as quickly as possible to a temperature at or above the ambient fluid (air) temperature, the controller 12 can be programmed to actuate solenoid 18 to cause vent door 14 to open and allow large quantities of ambient fluid to immediately flood the compartment 11 while heated fluid therein simultaneously escapes.

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Deactivation of the heating coil 31 while continuing activation of the blower with vent door 14 open, will draw ambient fluid into return compartment 34 and from there into the blower compartment 28. The blower will then push this ambient fluid through heating compartment 29 where it will pass directly into reaction compartment 30 without being heated by coil 31. The ambient fluid then passes through the sample compartment 27 and escapes out of chamber 11 through the vent door 14. Due to the minimum thermal mass of material located in chamber 11, and the action of the blower fan, vast quantities of ambient fluid will be forced past the sample compartment 27, and from there out of the chamber 11. Thus, rapidly cooling of samples or material located in the reaction compartment 27 is obtained.

The sample compartment 27 is sized so as to allow a plurality of samples, such as hollow elongate glass tubes containing a sample therein, to be easily located in a space apart orientation so that fluid may pass evenly around each sample. If desired, sample compartment 27 may be sized and configured so as to allow insertion of a rack, basket, or the

like which has been configured so as to accept a plurality of samples in uniform spaced apart configuration so as to simplify loading the samples into the sample chamber 27.

Access to sample compartment 27 is accomplished by rotation of the vent door 14 to its open position. Once the vent door 14 is rotated to approximately 90 degrees from it's closed position, the sample compartment 27 is easily accessible therethrough. Also, as can be seen, rotation of vent door 14 approximately 90 degrees from its closed position causes return fluid compartment 34 to be substantially closed off from reaction compartment 30. Thus, when the device 10 of the present invention is in a "cooling" mode, ambient fluid enters directly into return fluid compartment 34 and is forced through the blower compartment 28, heating compartment 29, reaction compartment 30, and sample compartment 27 substantially along the same path as the closed loop fluid flow path described above. The fluid is then forced out of the air chamber 11 and prevented from passing back into air return compartment 34 by the positioning of the vent door 14 between the sample compartment 27 and the return fluid compartment 34.

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Thus, the vent door 14 not only allows ambient fluid to enter the chamber 11, it can also prevent the fluid from recirculating in a loop fashion through the chamber 11. Instead, fluid is forced to pass through the sample compartment 27 and then out of the chamber 11 to aid in the rapid cooling of the sample contents and chamber 11.

When the device 10 of the present invention is used for cyclic DNA amplification, repetitive cycling through a temperature versus time profile is required. Samples containing a reaction mixture for the polymerase chain reaction generally must be cycled approximately 30 times through a temperature versus time profile which corresponds to the denaturation, annealing and elongation phases of the amplification process.

The device 10 of the present invention, due to its lower overall thermal mass, is capable of cycling samples through significantly shortened temperature versus time profiles compared to the prior art. The DNA amplification application of the embodiment of Figures 1-3 can pass through a temperature versus time profile cycle in 30-60 seconds (see Figure 5). This same cycle using prior art devices would take

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approximately 5-10 times longer. These low cycle times have proven also to increase yield and specificity of the polymerase chain reaction over prior art cycling.

EXAMPLE 1

The polymerase chain reaction was run in a 10 $\mu\ell$ volume with 50 ng template DNA, 0.5 mM of each deoxynucleotide 500 nM of each oligonucleotide primer in a reaction buffer consisting of 50 mM Tris-HCl (pH 8.5 at 25°C), 3.0 mM magnesium chloride, 20 mM HCl, and 500 μg/ml bovine serum albumin (5). Thermos aquatics polymerase (0/4 units of Taq polymerase - Stratagene™) was added, the samples placed in 8 cm long, thin-walled capillary tubes (manufactured by Kimble, Kimax 46485-1), and the ends fused with a laboratory gas burner so that an air bubble was present on both ends of each tube.

The capillary tubes were then placed vertically in a holder constructed of 1 mm thick "prepunched perfboard" (manufactured by Radio Shack). The mixture was cycled 30 times through denaturation (90-92°C), annealing (50-55°C), and elongation (72-75°C) for the times indicated in the temperature versus time profile of Figure 5. Temperature

monitoring of the capillary tubes was done with a miniature thermocouple (IT-23, Sensortek, Clifton, NJ) placed in 10 $\mu\ell$ of deionized water and connected to a thermocouple monitor (BAT-12, Sensortek). Amplification products were fractionated by electrophoresis on a 1.5% agarose gel. Good results were obtained.

Due to the fact that the device 10 of the present invention cycles air as the heat transfer medium instead of water, it has the advantage that heat transfer occurs through a low heat capacity medium (air) which can be warmed very rapidly.

The response time for sample cooling is very fast due to the use of thin walled glass capillary tubes for holding samples, instead of plastic microfuge tubes as has been done in the past with prior art processes, and by minimizing the thermal mass of material inside the chamber 11 (see Figure 5). Such response times can allow for optimization of the denaturation and annealing, and elongation steps in the polymerase chain reaction, in terms of time and temperature.

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Further, shortened "ramp" times are obtained, i.e., the time required to bring the temperature of the sample from one temperature level to the next temperature level corresponding to phases in the amplification process is shortened. This decreases the time required for a complete amplification, as well as allowing specific study of annealing, denaturation and enzyme kinetics within a polymerase chain reaction protocol.

The baffles 32 and 33 (as shown in Figure 3) may be used if desired to achieve adequate temperature homogeneity within the sample compartment 27. As shown in this embodiment, baffles 32 and 33 decrease the temperature variation in the reaction compartment 30 from about 10°C, to about 2°C. If desired, further (or more complicated) baffles may be used to further decrease the temperature variation in reaction compartment 30. Alternately, as shown in Figure 4 the fan may be positioned downstream from the heating coil 31, but before the sample compartment 27 to achieve more uniform mixing.

Amplification products obtained through the use of apparatus 10 are qualitatively and quantitatively similar to those obtained through the manual water bath cycling method. However, advantages in specificity and yield are possible with

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rapid thermal control of the reaction mixture. Figure 6 shows the effect of varying the denaturation time of the temperature versus time profile of Figure 5 as used with the thermal cycling apparatus 10 of the present invention on DNA amplification yields. The brighter vertical lines each correspond to a particular time at a denaturation temperature. As can be seen, the yield is greatest at the shortest possible denaturation time. Such a rapid response is not possible with prior art systems.

Figure 7 shows the effect of the temperature versus time profile of Figure 5 as used with the thermal cycling apparatus 10 on specificity (i.e., one specific product yield as opposed to a plurality of similar or "shadow" products). As can be seen, the shorter the ramp and annealing time, the greater the product specificity. The rapid temperature response of the apparatus 10 allows improved specificity and yield which is not possible with prior art systems.

As has been shown, by decreasing the thermal capacity (thermal mass) of the apparatus 10, the present invention can markedly decrease the total time required for the polymerase chain reaction. In addition, the use of small samples reduces

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the amounts of expensive reagents which must be used by up to about 90% thus further reducing the cost of carrying out procedures using the present invention. For example, capillary tubes 108 having inner diameters in the range from about 0.25mm to about 1.0mm can desirably be used. In some applications, capillary tubes 108 having inner diameters in the range from about 0.02mm to about 0.1mm can also be desirably used.

The apparatus 10 of the present invention is useful for amplifying DNA from any source. Although particular configurations and arrangements of the present invention have been discussed in connection with the specific embodiments of the thermal cycling device 10 as constructed in accordance with the teachings of the present invention, other arrangements and configurations may be utilized. For example, various fluids other than air, of generally low thermal mass, may alternatively be used in the device 10.

Another embodiment of the present invention is represented in Figures 8A-C. Figure 8A is a perspective view and Figure 8B is an elevational cross sectioned view of the additional embodiment. It will be understood that many of the

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earlier explained components and teachings also have application in the embodiment illustrated in Figures 8A-C. Thus, only the pertinent additional information concerning this embodiment will be provided below. Importantly, in the embodiment of Figures 8A-C, the heat producing element is adjacent to the biological sample containers allowing faster heating and cooling of biological samples as explained below.

As will be appreciated shortly, the apparatus of Figures 8A-C provides even greater improvement over the prior art in the speed at which thermal cycling can be carried out, e.g., 15 or 30 cycles of DNA amplification in 30, 15, 10 to 5, or even fewer, minutes. Furthermore, the apparatus 100 provides better thermal homogenization throughout the samples than previously possible.

Shown in Figure 8A is the general configuration of the housing 102 of the embodiment. The housing 102 rests on feet 104 (best seen in Figure 8B) and functions to hold the other described structures in place and to isolate those structures which become hot from the surrounding environment. Included in the embodiment 100 of Figure 8A are input keys 25 and a display 26 as in the previously described apparatus 10. The

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previously described control structures can readily be modified or used as a pattern for a control means for use in the embodiment of Figures 8A-C.

As shown best in the cross sectional view of Figure 8B, a sample chamber is designated by bracket 106. A lid 138 connected to the housing 102 by a hinge 131 can be opened to allow access to the sample chamber 106. The sample chamber 106 is preferably cylindrical in shape but can be of any shape or size required by the particular application.

The sample chamber 106 is lined with a black colored foam material 110 whose surface has light absorbing characteristics with the bulk of the thickness of the foam having insulating characteristics. The black foam material can be one which is readily available in the art and one fabricated from a plastic material. The foam 110 is preferably a material which is readily cooled by the air passing there over, i.e., the material has low thermal conductivity and a porous surface.

The dark or black porous surface of the material converts shorter wavelength radiation striking the surface into longer wavelength radiation, i.e., heat, which is radiated into the sample chamber.

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The foam 110 functions to thermally isolate the sample chamber from the surrounding air space in the housing and also to convert the light emitted by lamp 112 into thermal energy. The foam 110 can be replaced with other structures. For example, a material having a black, dark, or other nonreflective surface, such as a thin sheet of polycarbonate having one surface painted black, can be backed by an insulative material, such as a fiberglass or foam material. The black or dark surface, which can be painted on a number of different substrates, converts shorter wavelength radiation striking it into thermal radiation while the insulative material thermally isolates the sample chamber from the surrounding environment. Thus, using the teachings provided herein, those skilled in the art can utilize many different materials and structures as a lining for the sample chamber.

The lamp 112 is preferably a 500 watt halogen lamp. If appropriate control devices are used, higher power lamps or a plurality of lamps, such as four 500 watt halogen lamps, can be used. A lamp socket 112A is attached to the housing 102 by a support 112B. The lamp 112 is able to very rapidly and uniformly heat the sample chamber 106 to the desired

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temperature. Other sources of heat, i.e. infrared radiation, such as the earlier described nichrome wire element, can also be used within the scope of the present invention.

Represented in Figure 8B are two thin-walled capillary tubes 108 such as those described earlier. While two thin-walled capillary tubes 108 are shown, the sample chamber 106 can hold many such tubes. The thin-walled capillary tubes 108 have several important advantages over previously used devices as described earlier and, together with the sample chamber 106, function as the one presently preferred example of a means for holding a biological sample.

It will be appreciated that many other structures performing equivalent or similar functions can also be used. The thin-walled capillary tubes 108 are preferably left partially extending out of the sample chamber through apertures 140 for ease of access but may be completely contained within the sample chamber 106 as may numerous other fluid holding structures which are suited to particular applications. The preferred thin-walled capillary tubes 108 have a capacity of about 10 $\mu\ell$. As will be understood, the volume of the sample should be keep small, and the surface

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area of the sample holding structure relatively large, and together they should present a relatively small thermal mass. It is also preferred that the sample holding structure contain a volume anywhere from about 0.1 $\mu\ell$ to about 10,000 $\mu\ell$ but those skilled in the art will appreciate that other volumes of samples can also be used within the scope of the present invention if the different thermal mass of the structure is considered.

The lamp 112 and the insulative foam 110 together provide rapid and uniform heating of the sample contained in the thin-walled capillary tubes 108 and the air contained within the sample chamber 106. A thermocouple 134 is included within the sample chamber 106 to sense the temperature within the chamber and is used to maintain the desired temperature within the sample chamber as earlier described.

The thermocouple 134 is preferably one available in the art whose thermal response substantially matches the thermal response of the biological sample and the container holding the same. Such thermocouples can be commercially obtained from sources such as Idaho Labs which manufactures a line of thermocouples referred to as metal sheathed, J-type

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thermocouples. The matching of the thermal response of the thermocouple to that of the biological sample and container can be preferably carried out by inserting a micro thermocouple, such as the model IT-23 thermocouple available from PhysiTemp as known in the art, into a typical biological sample being held by the chosen container and subjecting the sample and the thermocouple under test to the same temperature changes. The thermocouple under test, or some external criteria, can be changed until the thermal response of the thermocouple suitably matches the thermal response of the sample and its container.

The arrangement represented in Figure 8B provides more uniform heating and cooling of the sample than previously available devices. In previously available devices, transfer of heat throughout the sample is carried out by convection through the sample. Convection induced movement of the sample within whatever structure is used to hold the sample is caused by temperature gradients or differences in the generally small biological samples (e.g., 10-100 $\mu\ell$).

The effect of temperature gradients within the sample become more pronounced and more difficult to control as the

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cycle time for a sample decreases. The existence of uneven temperatures within a sample, and particularly the reliance on "mixing by convection" within the sample by the prior art devices, generally increases the cycle time for a sample and likely has deleterious effects on the biological sample. The apparatus 100 is capable of providing heating and cooling such that thermal differences within a 10 $\mu\ell$ sample are maintained at not greater than $\pm 1^{\circ}C$ at all times during a 30 second cycle.

In order to promote uniform heating and cooling, it is preferred that the thin-walled capillary tubes 108 be at least somewhat uniformly spaced from the heat source, for example, lamp 112 in apparatus 100. Figure 8C provides a diagrammatic top view of the lamp 112 and the plurality of thin-walled capillary tubes 108 as arranged in the apparatus 100 represented in Figures 8A-B.

In the arrangement represented in Figure 8C, the thin-walled capillary tubes 108 which are farthest from the lamp 112 (as indicated by line F) are preferably no more than substantially 40%, and more preferably no more than substantially 25%, farther from the lamp 112 than the distance

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between the lamp 112 and those thin-walled capillary tubes 108 which are closest to the lamp 112 (as indicated by line N). For example, the distance indicated by line N can be about 7.3 cm while the distance indicated by line F can be about 8.5 cm.

It will be appreciated that the arrangement of the thin-walled capillary tubes 108 can be other than that represented in the figures, for example, circular or semi-circular. Moreover, it will appreciated that the point from which to measure the distance between the heat producing element and the sample containers will vary as the type and size of the heat producing element varies. For example, the heat producing element may comprise a plurality of lamps or electric resistive elements which vary in shape and size. In some embodiments, it may also become important to consider the distance from the sample chamber wall the sample containers are positioned. In the illustrated embodiment, the apertures 140 (see Figure 8A) function as a means for holding the sample containers but other structures performing equivalent functions can also be used in accordance with the present invention.

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The apparatus 100 also cools the samples contained in the capillary tubes 108 very rapidly and uniformly. In order to cool the sample chamber 106, air from outside the housing 102 is draw into the interior of the housing through a lower housing portal 114 by a fan 116 which is connected to a motor shaft 122 driven by a motor 118. Since rapid cooling of the sample chamber is desired, it is preferred that the combination of the motor 118 and the fan 116 be able to move sufficient volumes of air into the sample chamber 106 and then disperse that air inside the sample chamber 106, as will be explained shortly. Arrangements other than the motor 118 and fan 116 illustrated in Figure 8B can also be used within the scope of the present invention.

The use of air as the thermal transfer medium, in contrast to other gases and liquids, has the advantages of being inexpensive, readily available, easily mixed, and never making a mess. In the case of the described embodiments, the high surface area-to-volume ratio of the sample containing capillary tubes provides for rapid thermal transfer using air as the thermal transfer medium.

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During cooling portions of the thermal cycle, the action of the fan 116 draws ambient temperature air into the housing 102. A vent door 128, articulating on hinge 129, is provided. The vent door 128 is automatically opened by way of a solenoid 132 so that the interior of the housing 102 is sealed off from the upper housing portal 130. In some embodiments, the solenoid 132 is preferably replaced by a stepper motor as is known in the art. The use of a stepper motor allows the vent door 128 to be accurately and incrementally opened and closed in accordance with the needs for heating and cooling the samples. Those skilled in the art will be able to derive an appropriate control mechanism for use with a stepper motor, for example an SC-149 stepper motor controller (available from Alpha Products) as known in the art, using the information set forth herein.

Due to the arrangement of the lower sample chamber portal 120 and the larger cross sectional area and position of the upper sample chamber portal 126, room temperature air is moved into the sample chamber 106 and is dispersed and mixed within the sample chamber 106 by a paddle 124 which is connected to the motor shaft 122. The paddle 124 should rotate at a

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relatively high rate, for example, fast enough to create air velocities of around preferably about 250, more preferably 500, and most preferably 1000 meters per minute within the sample chamber 106. With the paddle 124, which can be a single or a multivane paddle, rotating at a high speed, air is moved, or drawn, into the sample chamber 106 and vented out of the sample chamber 106 following the path indicated by the dashed line 136. The rotation of the paddle 124 also promotes mixing of the air entering the sample chamber 106 and ensures the most efficient transfer of thermal energy from the surfaces of the thin-walled capillary tubes 108 to the air passing through the sample chamber 106. It will be appreciated that structures other than those illustrated herein can perform equivalent functions.

As the solenoid 132 is actuated to open the vent door 128, all of the room temperature air moved into the sample chamber 106 is exhausted through a sample chamber upper portal 126 and then through the upper housing portal 130 carrying the heat from the sample chamber 106 to the surrounding atmosphere. The rapid mixing of the air that passes through,

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and is disbursed in, the sample chamber 106 results in rapid and uniform cooling of the samples.

Example 2

Figure 9A shows the results of four different temperature/time profiles (A-D) and their resultant amplification products after thirty cycles (A-D). The profiles A and B in Figure 9A were obtained using a prior art heating block device using the prior art microfuge tube. As can be seen in Figure 9A, the transitions between temperatures are slow and many nonspecific bands are present in profiles A and B. Profile B shows improvement in eliminating some of the nonspecific bands (in contrast to profile A) by limiting the time each sample remains at each temperature thus indicating that shorter times produce more desirable results.

Profiles C and D were obtained using the apparatus of Figures 8A-B. As can be seen in Figure 9A, amplification is specific and, desirably, even though yield is maximal in C (60 second elongation) it is still entirely adequate in D (10 seconds elongation).

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The optimal times and temperatures for the amplification of a 536 bp fragment of β-globin from human genomic DNA were also determined. Amplification yield and product specificity were optimal when denaturation (93°C) and annealing (55°C) were less than 1 second. No advantage was found to longer denaturation or annealing times. The yield increased with longer elongation times at (77°C) but there was little change with elongation times longer than 10-20 seconds. These unexpected results indicate that the previously available devices used for DNA amplification are not maximizing the conditions needed to optimize the physical and enzymatic requirements of the reaction.

Further information can be obtained from: Wittwer, Carl T., Marshall, Bruce C., Reed, Gudrun B., and Cherry, Joshua L., "Rapid Cycle Allele-Specific Amplification with Cystic Fibrosis ΔF_{508} Locus," 39 Clinical Chemistry 804 (1993) and Wittwer, Carl T., Reed, Gudrun H., and Rire, Kirk M., "Rapid DNA Amplification," THE POLYMERASE CHAIN REACTION 174 (1994) which are both now incorporated herein by this reference.

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From the information provided in Figure 9A, it can be seen that the embodiments of the present invention subject the samples placed therein to rapid thermal cycling wherein the temperature of the sample is increased and decreased at a rate preferably at least as great as 0.5°C/second. In the case of the present invention carrying out the polymerase chain reaction, the temperature change is preferably carried out over an approximate range of between 30°C to 50°C. It is preferred that the thermal cycles be carried out quickly enough to complete at least thirty thermal cycles in forty minutes and more preferably complete thirty thermal cycles in twenty minutes and most preferably complete thirty thermal cycles in ten minutes.

The apparatus 100 more preferably increases and decreases the temperature of the sample at a rate at least as great as 1.0°C/second and even more preferably at a rate at least as great as 4.0°C/second and most preferably at a rate at least as great as 10.0°C/second. Critically, the biological sample, not just the surrounding medium and/or the sample container, must undergo the specified thermal change. The previously available devices, while having the drawback of not able to

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perform thermal changes as rapidly as the present invention, also did not recognize the problem of changing the temperature of the sample, not just the temperature of the surrounding medium and container, rapidly and uniformly.

Referring now to the chart of Figure 9B, the method of the present invention can desirably achieve thermal cycling preferably at a rate at least as great as 10°C/sec., and more preferably at a rate at least as great as 20°C/sec., over a temperature range of greater than about 20°C, more preferably over a temperature range of greater than about 30°C, and most preferably over a temperature range of about 40°C. Figure 9B shows the temperature in °C of the biological sample, not just the surrounding air or container, as the biological sample undergoes the thermal cycle. Figure 9B shows a PCR sample beginning at about 74°C and being heated to a denaturation temperature, indicated at D, of about 92°C for 2 seconds. The sample is then cooled to an annealing temperature, indicated at A, of about 55°C for two seconds. The transition between the denaturation temperature and the annealing temperature covers a range of 37°C in just under 4 seconds providing a rate at least as great as 10°C/sec. The sample is then warmed

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to an extension temperature of 74°C for five seconds as indicated at E in Figure 9B. The cycling of the sample through the denaturation temperature, the annealing temperature, and the extension temperature is repeated thirty time or as many times as desired.

Figures 9C-G show exemplary cycles of other preferred temperature/time profiles which are achieved by the present invention. It will be understood that those skilled in the art can alter the represented temperature/time profiles to carry out specific processes in accordance with the present invention. Those skilled in the art will also appreciate that the previously available devices and methods, such as devices which conduct heat to and from the sample via a solid or liquid, cannot provide, and do not suggest or teach, the temperature/time profiles described herein. Furthermore, it will be appreciated that the previously available devices and methods utilizing air and the transfer medium, for example previously available chromatographic ovens, cannot provide, and do not suggest or teach, the temperature/time profiles described herein.

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In order to provide the fastest thermal cycling time, it is preferred that the lamp (112 in Figures 8A and 8B) be rated at 2000 watts or a plurality of lamps be included which provide similar output. It is also preferred to include a temperature slope control circuit which is represented in Figure 10 in conjunction with an A-bus controller/acquisition system using an 8052 micro controller board with a clock and high level program interpreter available from Alpha Products (model no. SP-127) of Darian, Connecticut. Exemplary programming code used in connection with the described microcontroller is included in the Programming Code Appendix attached hereto and incorporated herein. The programming code provided in the Appendix is a BASIC52 file for serial downloading into the microcontroller and provides exemplary temperature slope control during thermal cycling. Use of the 2000 watt heat producing device and the describe control structures allows rates of 20°C/sec. to be desirably obtained.

The preferred arrangement for the temperature slope control circuit represented in Figure 10 will be explained with the understanding the additional necessary components not

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explicitly illustrated in Figure 10 can readily be supplied by those skilled in the art.

The temperature slope control circuit of Figure 10 includes a thermocouple 200 matched to the sample temperature response as explained earlier. The thermocouple 200 is connected to an integrated circuit 206, which preferably is one known in the art as an AD595, whose output is conveyed to a 4th order low pass filter 208 with a cutoff frequency of 100 Hz and to a 12 bit analog-to-digital convertor 210 whose output is used to provide a digital display of the temperature.

The output of the circuit 206 is also conveyed to a measured slope circuit 212. The measured slope circuit 212 preferably includes a 353 operational amplifier 218, a 100 KΩ potentiometer 214, a 1 MΩ potentiometer 230, and a 22 μF capacitor. The measured slope circuit 212 outputs a signal to the inverting input of a 353 operational amplifier 246.

A slope set circuit 222 includes a positive slope set digital-to-analog converter 226 and a negative slope set digital-to-analog converter 224. The digital-to-analog converters 224 and 226 are preferably 8-bit digital-to-analog

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converters referred to in the art as DA147. The slope set circuit can preferably receive instructions from another digital device (not illustrated in Figure 10) such as a personal computer. The output of the slope set circuit 228 is communicated to a summing circuit 240.

The summing circuit 240 preferably includes 100 KΩ resistors 236, 238, and 244 and a 353 operational amplifier 242. The output of the summing circuit 240 is conveyed to the non-inverting input of the operational amplifier 246 and represents the desired slope of the temperature change. The output of the operational amplifier 246 is provided to a transistor 248 contained within a power switching circuit 262.

The power switching circuit 262 includes a 5 VDC supply 250 providing current to the transistor 248. The transistor 248 has its emitter connected to a 3010 circuit 254 by way of resistor 252 which is preferably a 330 Ω resistor. The 3010 circuit 254 includes an output connected in series with a resistor 256 which preferably is a 180 Ω resistor. A triac 258 is preferably used to control the current delivered to a lamp 262, or other heat producing device, from a source of AC current 260.

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The temperature slope control circuit represented in Figure 10, in cooperation with the other described system components, provides thermal cycling of biological samples as great as 20°C/sec over a temperature range of 30°C, and most preferably over a temperature range of 40°C, with homogeneity being maintained throughout the biological sample.

It will be appreciated that the apparatus described herein can readily be used for many different applications including: polymerase chain reaction processes; cycle sequencing; and, other amplification protocols such as the ligase chain reaction. The present invention also advantageously provides an apparatus for accurately controlling the temperature of samples located in the sample chamber and quickly and accurately varies the temperature of samples located in a chamber according to a predetermined temperature versus time profile.

The present invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore,

indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

What is claimed and desired to be secured by United States Letters Patent is:

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1. A method of subjecting at least a first biological sample to rapid thermal cycling, the method comprising the steps of:

(a) placing the first biological sample in at least a first container;

(b) raising the temperature of the first biological sample from a first temperature to a second temperature at a first rate at least as great as 1°C per second, the first temperature being at least 20°C different than the second temperature;

(c) holding the first biological sample at a temperature at least as great as the second temperature for not more than a first holding period, the first holding period being not greater than ten seconds;

(d) lowering the temperature of the first biological sample from the second temperature to at least the first temperature at a second rate at least as great as 1°C per second;

(e) holding the first biological sample at a temperature at least as low as the first temperature for

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not more than a second holding period, the second holding period being not greater than ten seconds; and

(f) removing the first biological sample from the first container.

2. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the first temperature is at least 30°C different than the second temperature.

3. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the first temperature is at least 40°C different than the second temperature.

4. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the first rate is at least as great as 4°C per second.

5. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 4 wherein the second rate is at least as great as 4°C per second.

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6. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the first rate is at least as great as 10°C per second.

7. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 6 wherein the second rate is at least as great as 10°C per second.

8. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the first holding period is not greater than 3 seconds.

9. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 8 wherein the second holding period is not greater than 3 seconds.

10. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the first holding period is not greater than 1 second.

11. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 10 wherein the second holding period is not greater than 1 second.

12. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein

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the step of placing the first biological sample in at least a first container comprises the steps of:

placing a first portion of the biological sample into the first container;

placing a second portion of the biological sample into a second container;

placing a third portion of the biological sample into a third container; and

placing a fourth portion of the biological sample into a fourth container.

13. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 12 further comprising the step of positioning the first, second, third, and fourth containers in a sample chamber and wherein the step of raising the temperature of the first biological sample comprises the step of subjecting the first, second, third, and fourth containers to infrared radiation.

14. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 further comprising the step of repeating steps (b) through (e) at least fifteen times.

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15. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 further comprising the step of repeating steps (b) through (e) at least thirty times in not more than 10 minutes.

16. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of placing the first biological sample in at least a first container comprises the step of placing the first biological sample in at least the first container having a volume in the range from about 1 $\mu\ell$ to about 10,000 $\mu\ell$.

17. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 16 wherein the step of placing the first biological sample in at least a first container further comprises the step of placing the first biological sample in at least the first container having an inner diameter in the range from about 0.02mm to about 1.0mm.

18. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of raising the temperature of the first biological sample comprises the step of adjusting the first rate.

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19. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of lowering the temperature of the first biological sample comprises the step of adjusting the second rate.

20. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of holding the biological sample at a temperature comprises the step of adjusting the length of the first holding period.

21. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the step of lowering the temperature of the first biological sample comprises the step of moving ambient air past the first container at a rate at least as great as 500 meters per minute.

22. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 1 wherein the first biological sample comprises DNA and primers.

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23. A method of subjecting at least a first biological sample to rapid thermal cycling, the method comprising the steps of:

(a) placing the first biological sample in at least a first container, the first container having a volume in the range from about 1 $\mu\ell$ to about 10,000 $\mu\ell$;

(b) raising the temperature of the first biological sample from a first temperature to a second temperature at a first rate at least as great as 10°C per second, the first temperature being at least 30°C different than the second temperature, the temperature of the first biological sample being homogeneously raised such that no portion of the first biological sample is more than about 5°C warmer or cooler than any other portion of the first biological sample;

(c) holding the first biological sample at a temperature at least as great as the second temperature for not more than a first holding period, the first holding period being not greater than three seconds;

(d) lowering the temperature of the first biological sample from the second temperature to at least

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the first temperature at a second rate at least as great as 10°C per second;

(e) holding the first biological sample at a temperature at least as low as the first temperature for not more than a second holding period, the second holding period being not greater than three seconds;

(f) removing the first biological sample from the first container; and

(g) repeating steps (b) through (e) at least fifteen times.

24. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 23 further comprising the step of repeating steps (b) through (e) a plurality of times and wherein the step of raising the temperature of the first biological sample comprises the step of holding the temperature of the first biological sample at a third temperature for not more than 10 seconds, the third temperature being intermediate the first temperature and the second temperature.

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25. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 23 wherein the step of placing the first biological sample in at least a first container comprises the steps of:

placing a first portion of the biological sample into the first container;

placing a second portion of the biological sample into a second container;

placing a third portion of the biological sample into a third container; and

placing a fourth portion of the biological sample into a fourth container;

and wherein the method further comprises the step of positioning the first, second, third, and fourth containers in a sample chamber and wherein the step of raising the temperature of the first biological sample comprises the step of subjecting the first, second, third, and fourth containers to infrared radiation.

26. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 23 wherein

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the step of raising the temperature of the first biological sample comprises the step of adjusting the first rate.

27. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 23 wherein the step of lowering the temperature of the first biological sample comprises the step of moving ambient air past the first container at a rate at least as great as 500 meters per minute.

28. A method of subjecting at least a first biological sample to rapid thermal cycling as defined in claim 23 wherein the first biological sample comprises DNA and primers.

29. A method of subjecting at least a first biological sample containing a DNA template, an oligonucleotide primer, and a reaction buffer to perform cyclic DNA amplification using a thermostable DNA polymerase, the method comprising the steps of:

(a) placing the first biological sample in a plurality of containers, each container having a volume in the range from about 1 $\mu\ell$ to about 10,000 $\mu\ell$;

(b) raising the temperature of the first biological sample placed in each of the plurality of containers from

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a first temperature to a second temperature at a first rate at least as great as 10°C per second, each of the plurality of containers being subjected to infrared radiation, the first temperature being at least 30°C different than the second temperature, the temperature of the first biological sample being homogeneously raising such that no portion of the first biological sample is more than about 5°C warmer or cooler than any other portion of the first biological sample found in the same or any of the other of the plurality of containers;

(c) holding the first biological sample at a temperature at least as great as the second temperature for not more than a first holding period, the first holding period being not greater than one second;

(d) lowering the temperature of the first biological sample from the second temperature to at least the first temperature at a second rate at least as great as 10°C per second by moving air past the first container at a rate at least as great as 250 meters per minute;

(e) holding the first biological sample at a temperature at least as low as the first temperature for

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not more than a second holding period, the second holding period being not greater than one second;

(f) removing the first biological sample from substantially all of the plurality of containers; and

(g) repeating steps (b) through (e) at least fifteen times.

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ABSTRACT OF THE DISCLOSURE

A thermal cycling method and device is disclosed. The device comprises a sample chamber whose temperature can be rapidly and accurately modulated over a range of temperatures needed to carry out a number of biological procedures, such a the DNA polymerase chain reaction. Biological samples are placed in glass micro capillary tubes and then located inside the sample chamber. A programmable controller regulates the temperature of the sample inside the sample chamber. Once a heating cycle is completed, the controller opens a door to the chamber for venting hot air out and cool ambient air is moved in. Temperature versus time profiles corresponding to optimum denaturation, annealing and elongation temperatures for amplification of DNA are achieved by the present invention.

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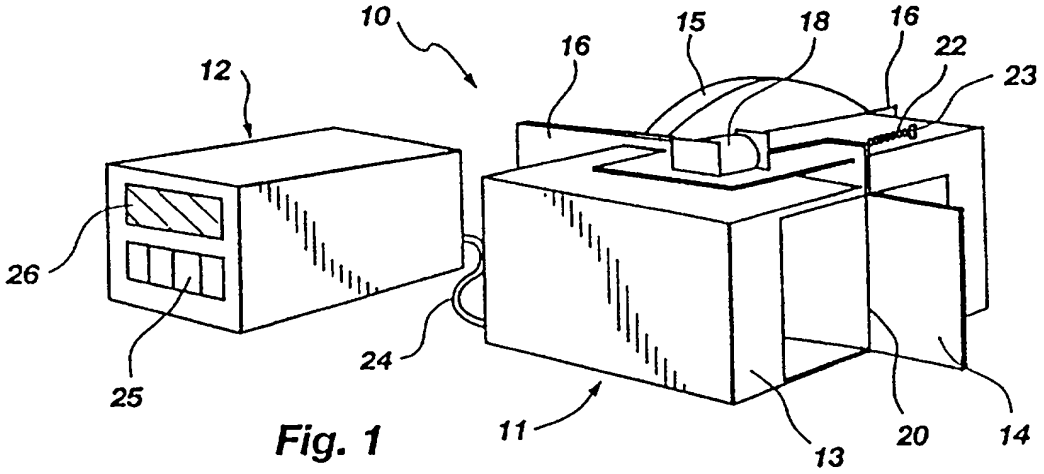


Fig. 1

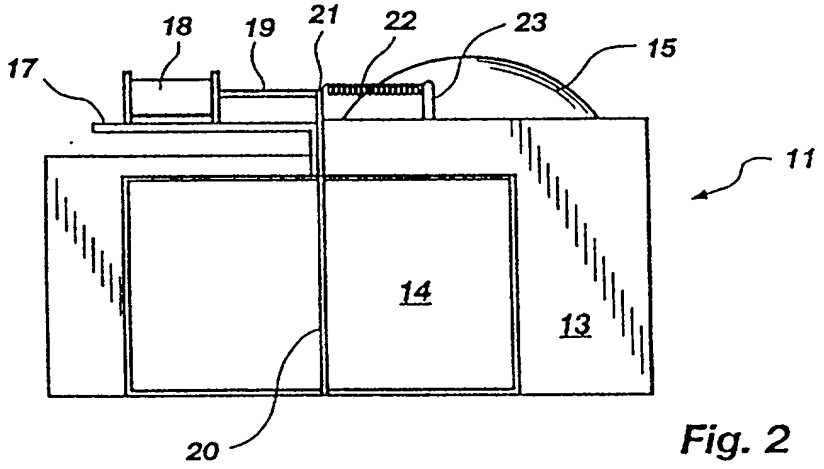


Fig. 2

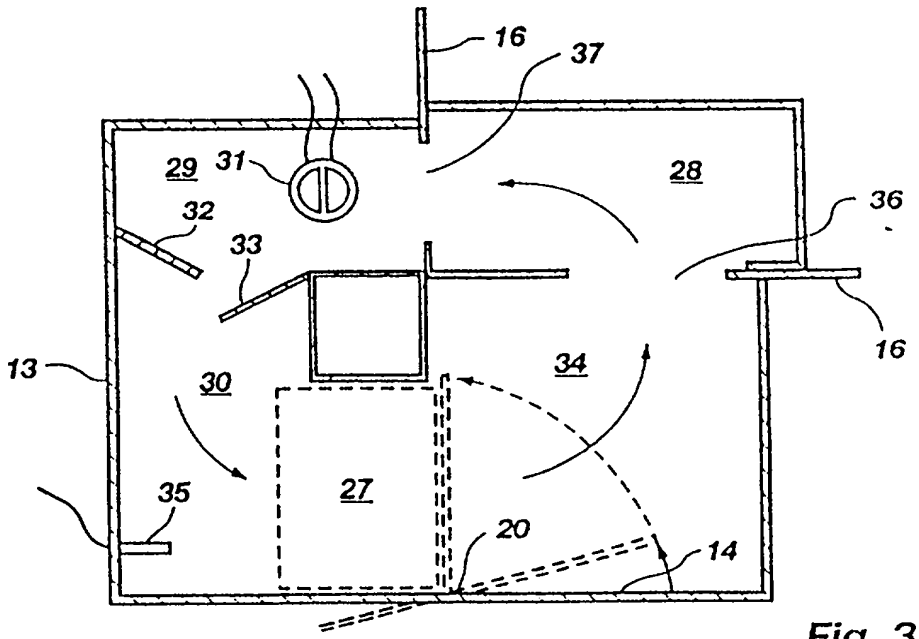


Fig. 3

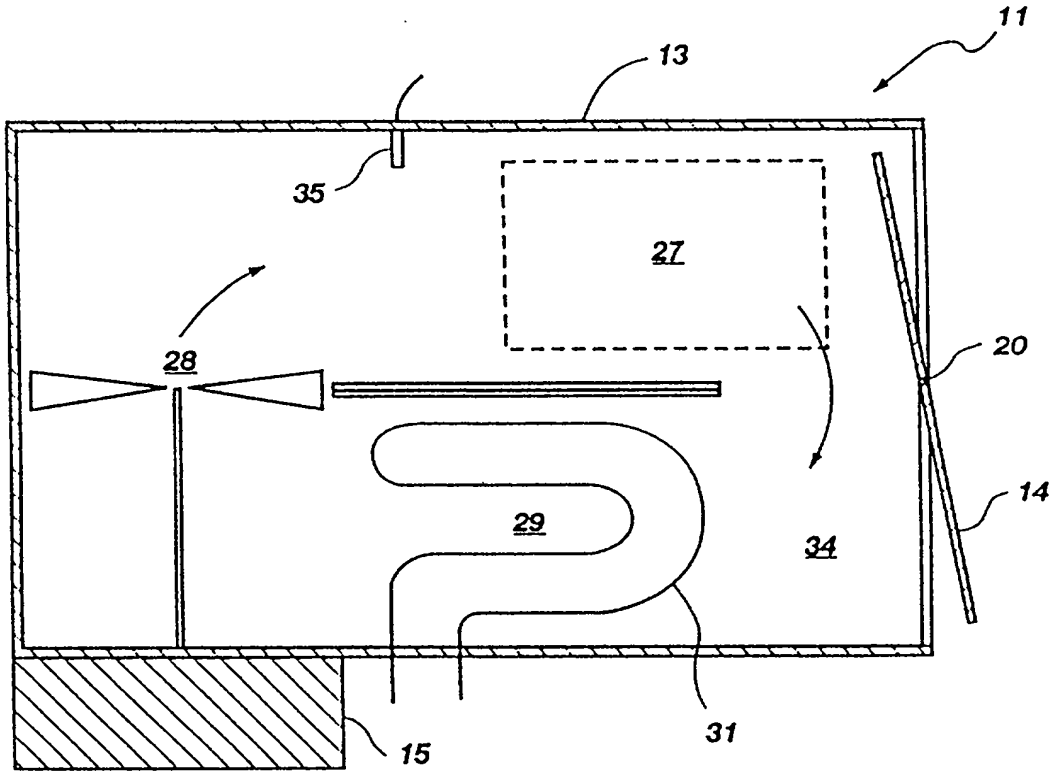

11
13
35
27
28
20
14
29
34
31
15

Fig. 4

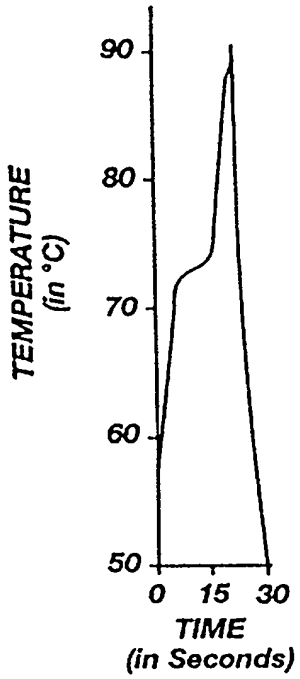
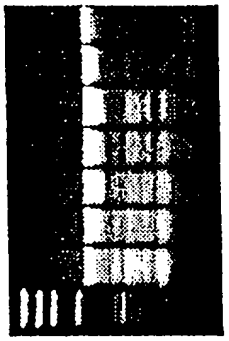

90
80
70
60
50
TEMPERATURE
(in °C)
0
15
30
TIME
(in Seconds)

Fig. 5

Effect of Annealing Time on Product Yield and Background Amplification

92 to 55 °C Ramp Time (sec)	Time at 55 °C (sec)
9	<1
25	<1
25	5
25	10
25	20
25	40
25	80



PhiX174 RF Hae III Digest

Fig. 7

Effect of Denaturation Time on Product Yield

Time at 92-94 °C (sec)
<1
2
4
8
16
32
64

PhiX174 RF Hae III Digest

Fig. 6

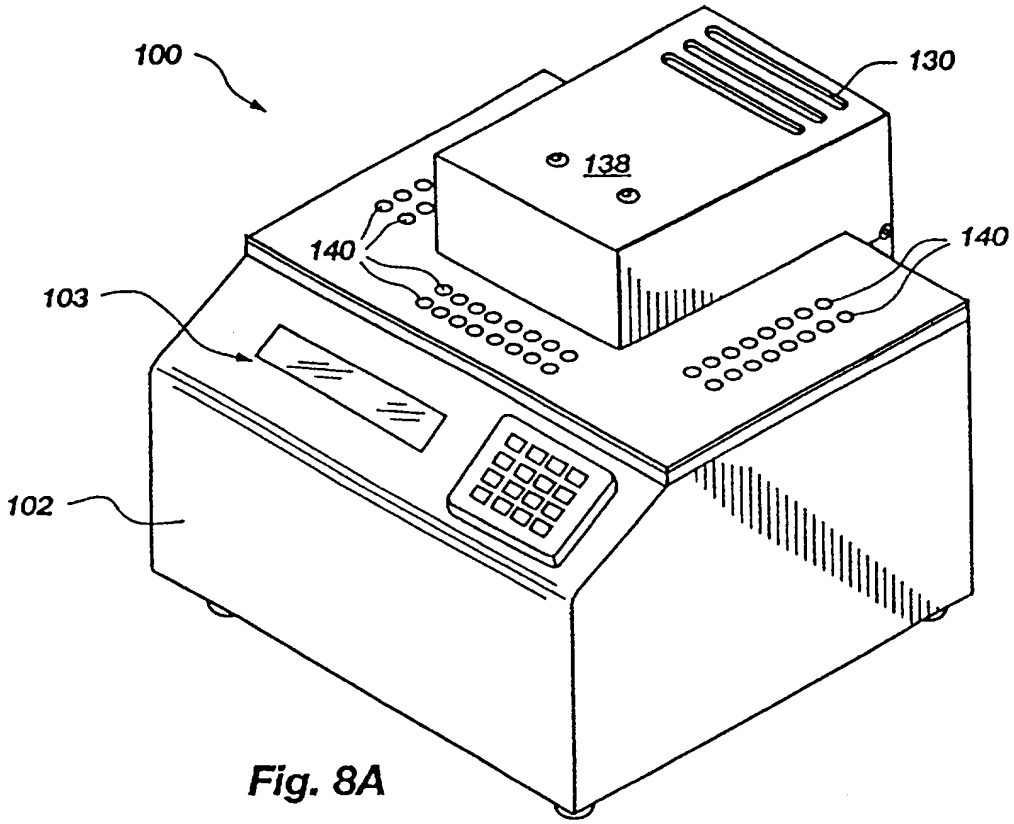
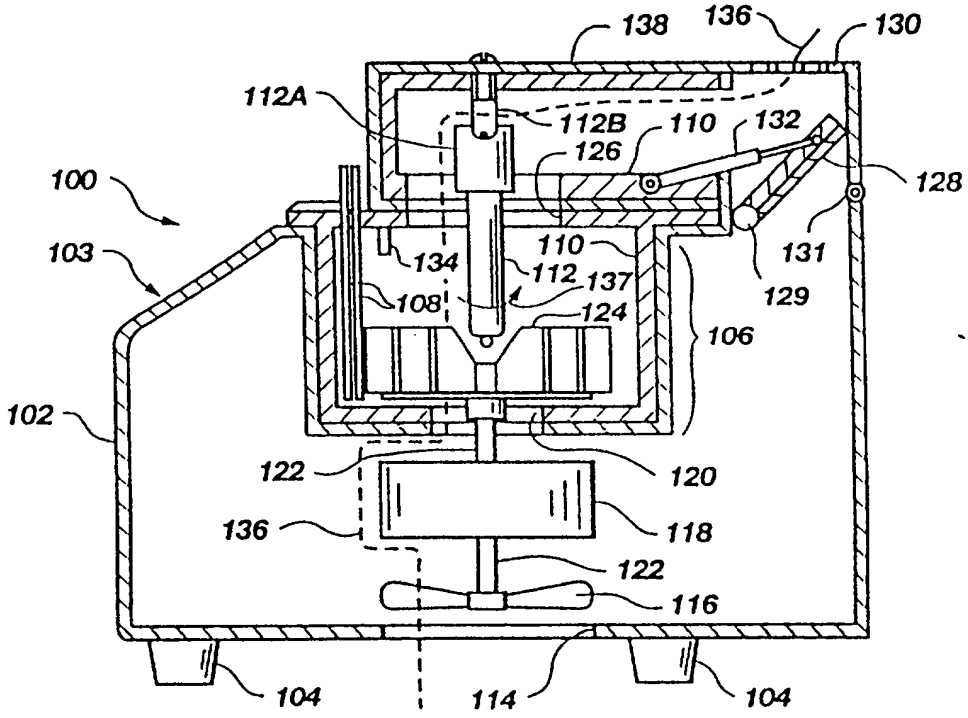

100
130
138
140
140
103
102

Fig. 8A


138
136
130
112A
112B
110
132
126
128
100
103
134
110
112
137
131
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102
122
120
136
118
122
116
104
114
104

Fig. 8B

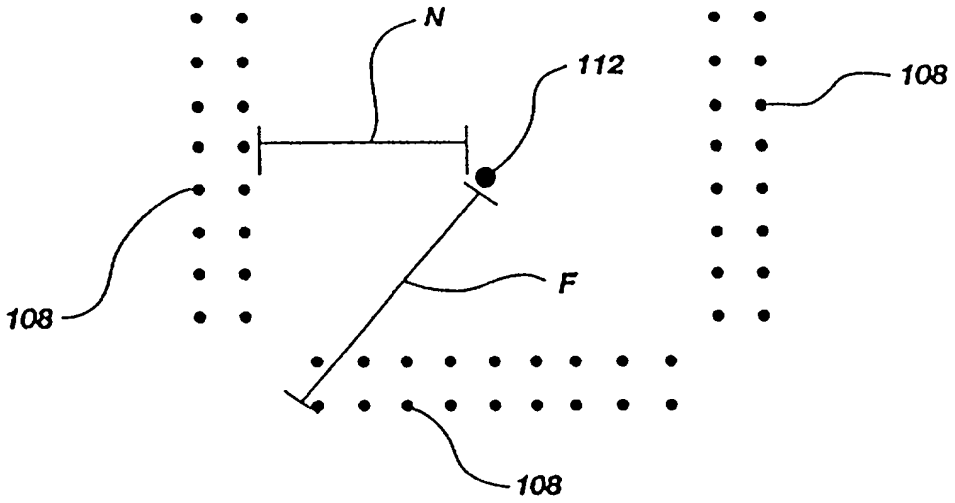


Fig. 8C

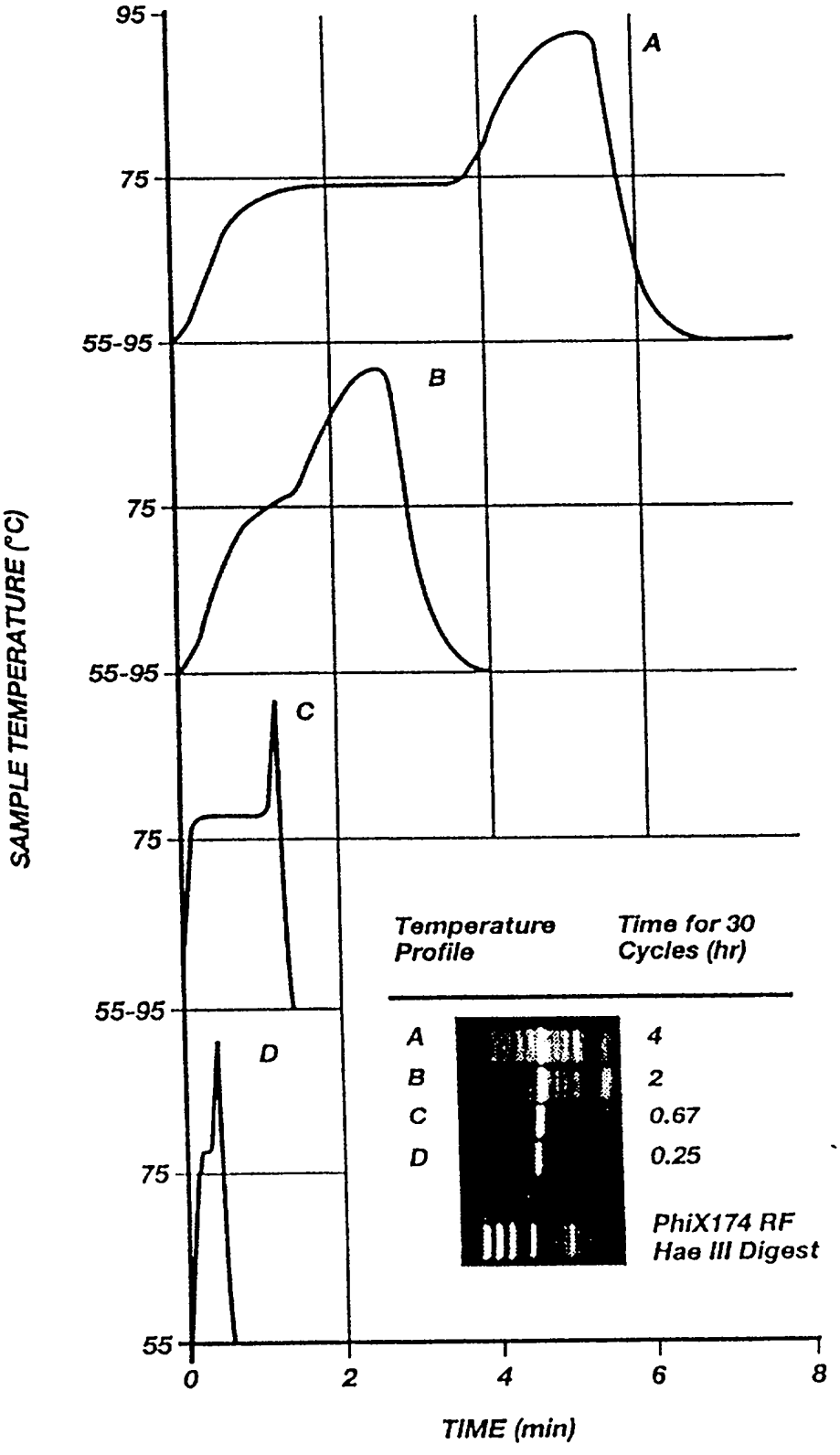

95
75
55-95
75
55-95
75
55-95
75
55
A
B
C
D
SAMPLE TEMPERATURE (°C)
0
2
4
6
8
TIME (min)
Temperature Profile
Time for 30 Cycles (hr)
A
B
C
D
4
2
0.67
0.25
PhiX174 RF Hae III Digest

Fig. 9A

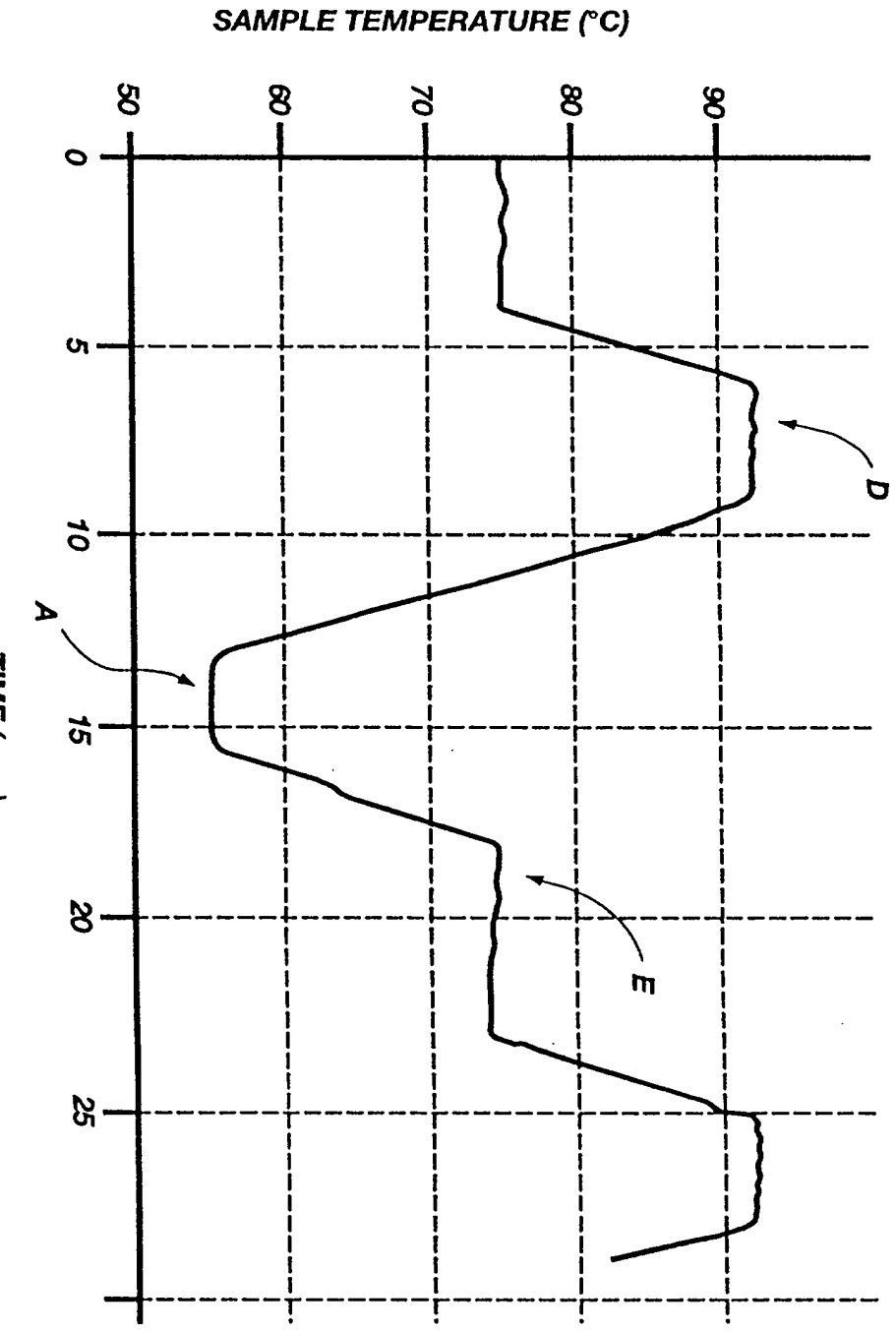


Fig. 9B

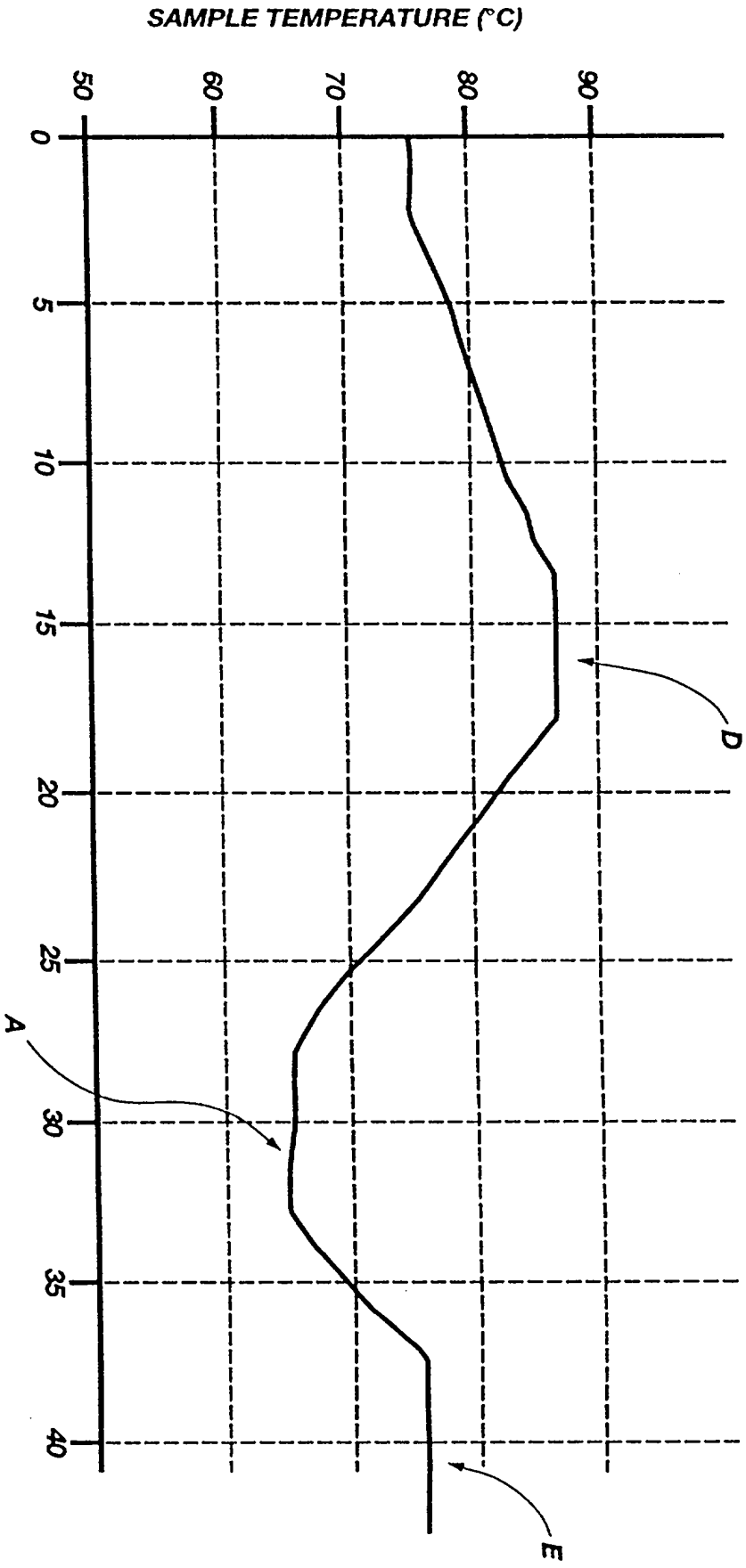
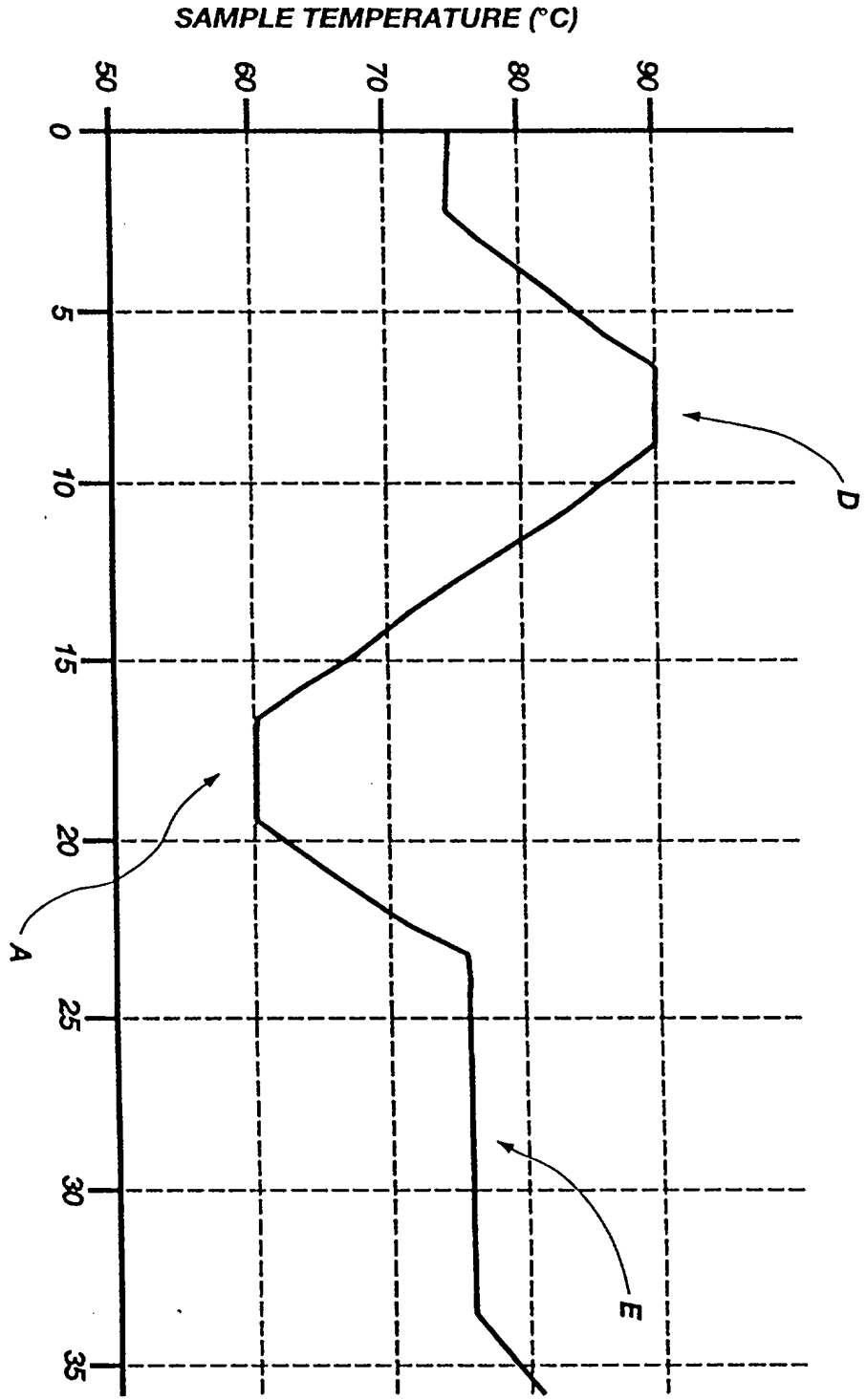

SAMPLE TEMPERATURE (°C)
50
60
70
80
90
0
5
10
15
20
25
30
35
40
TIME (sec.)
A
D
E

Fig. 9C


SAMPLE TEMPERATURE (°C)
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60
70
80
90
0
5
10
15
20
25
30
35
TIME (sec.)
A
D
E

Fig. 9D

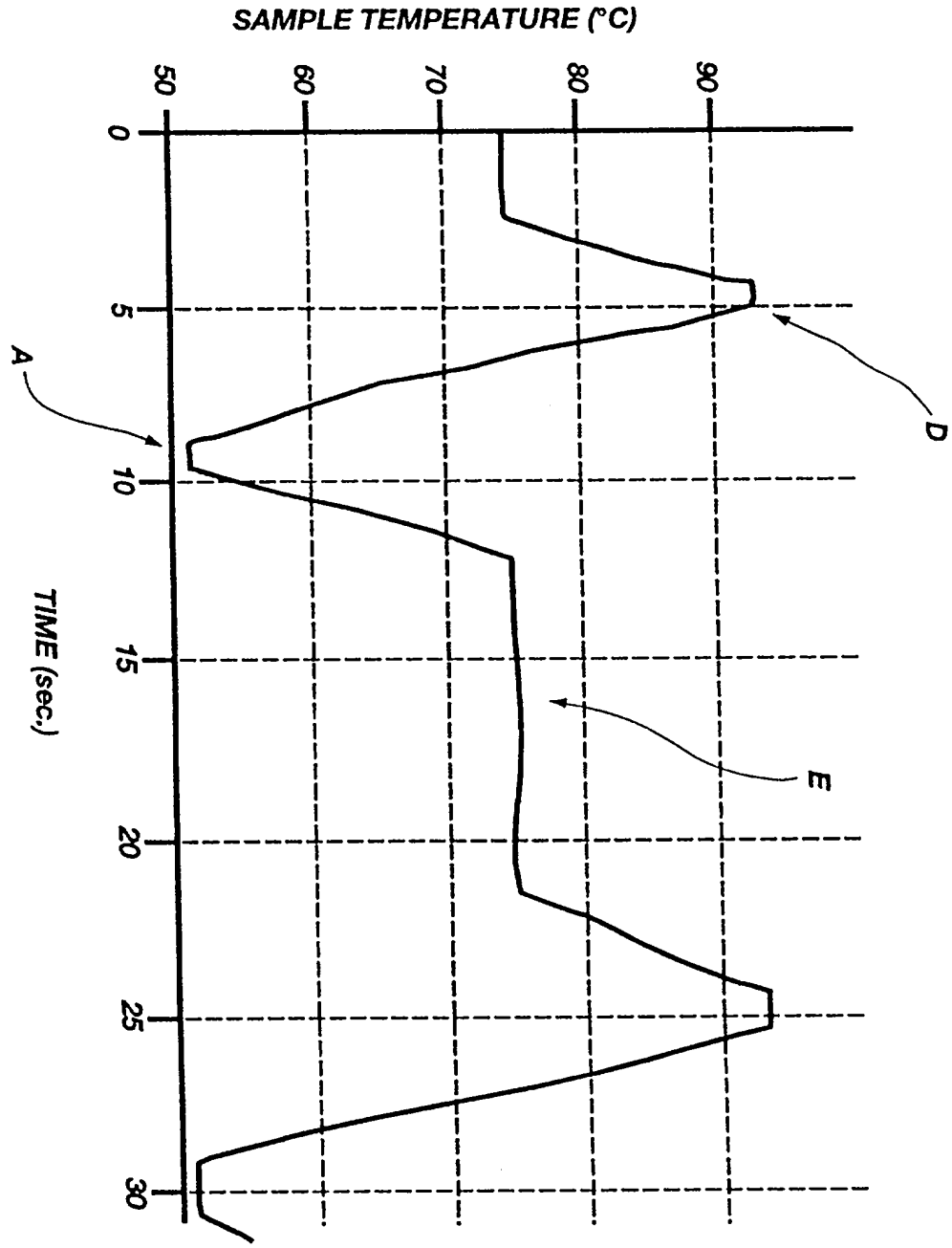
SAMPLE TEMPERATURE (°C)
50
60
70
80
90
0
5
10
15
20
25
30
TIME (sec.)
A
D
E

Fig. 9E

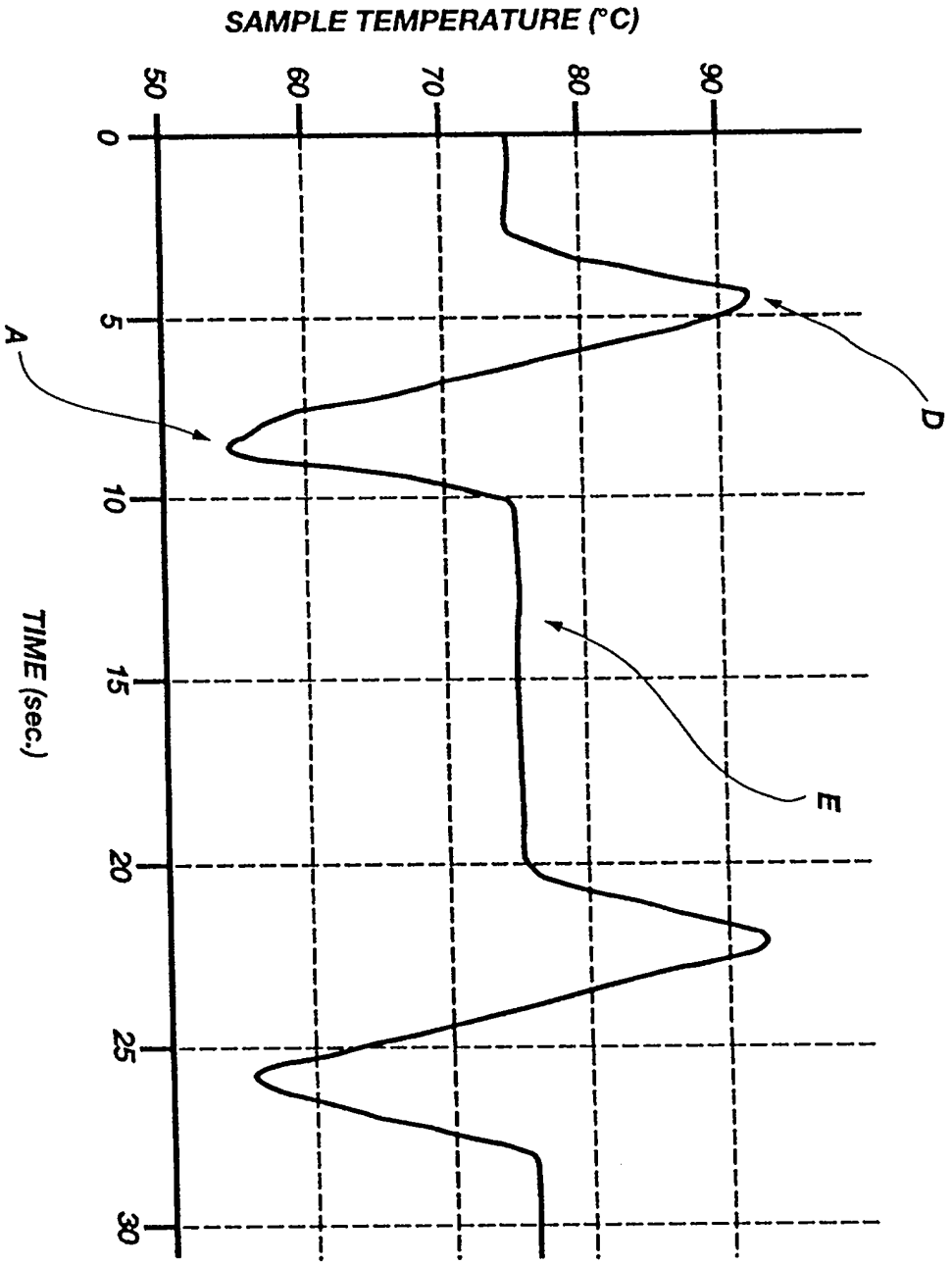
SAMPLE TEMPERATURE (°C)
50
60
70
80
90
0
5
10
15
20
25
30
TIME (sec.)
A
D
E

Fig. 9F

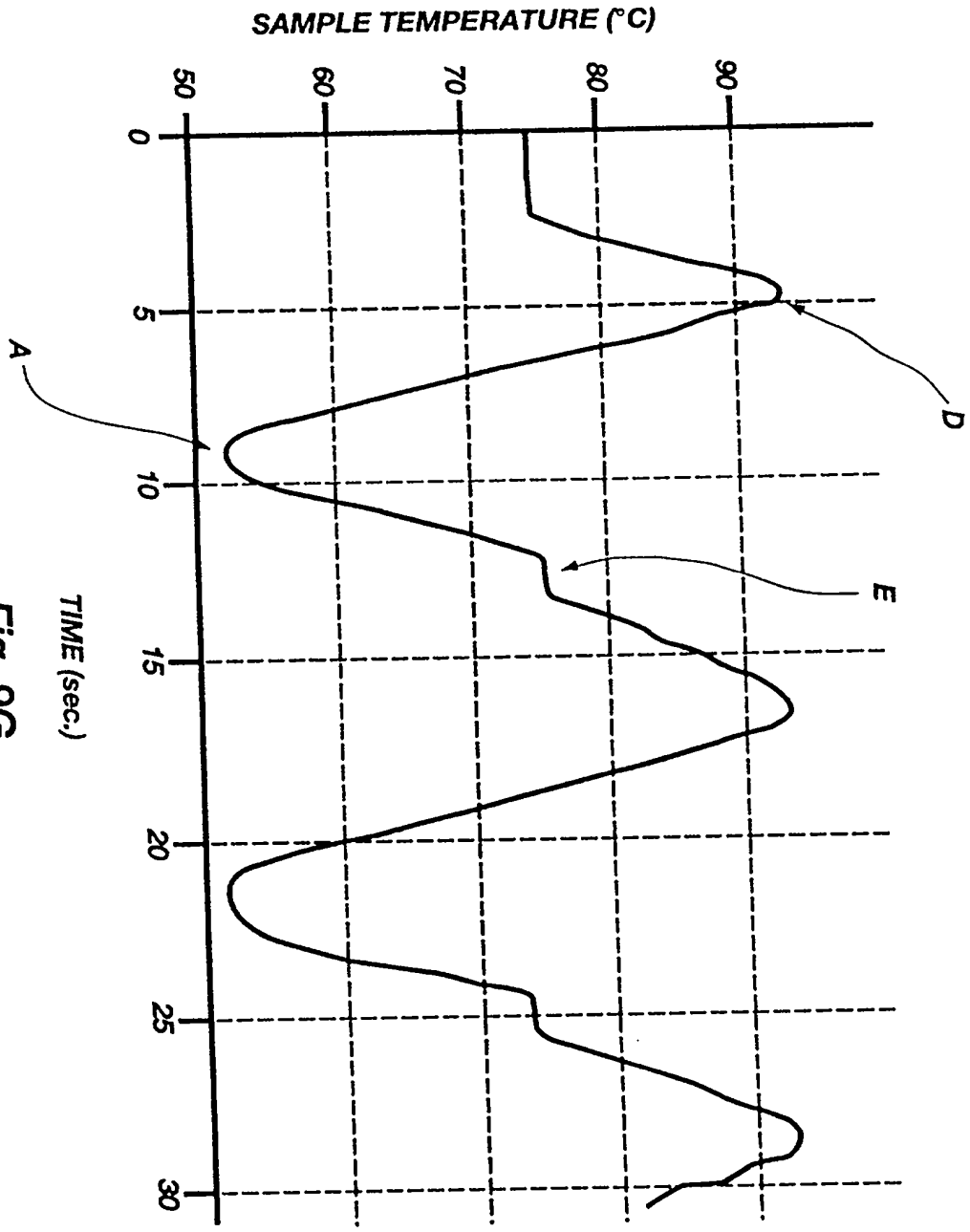
SAMPLE TEMPERATURE (°C)
50
60
70
80
90
0
5
10
15
20
25
30
TIME (sec.)
A
D
E

Fig. 9G

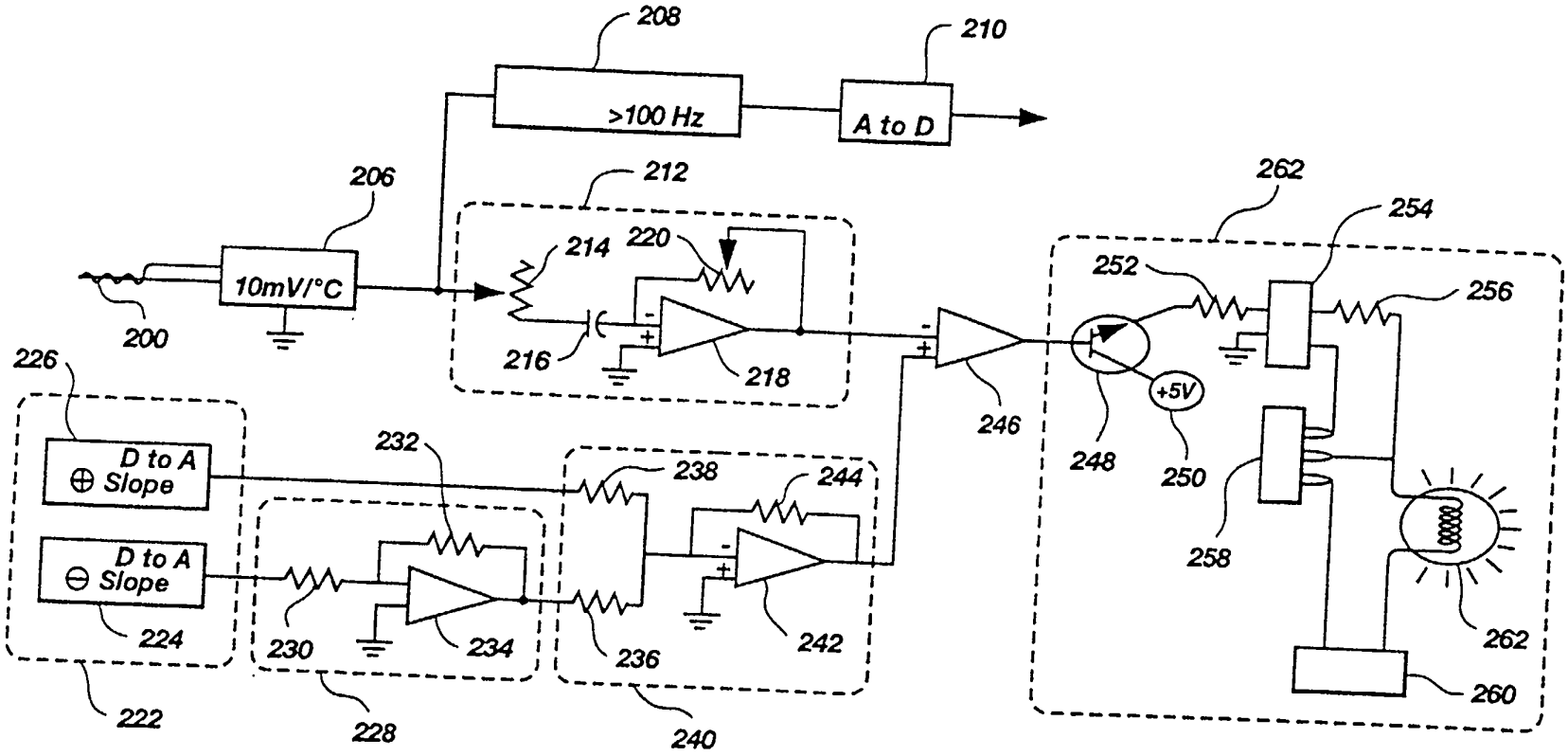

Fig. 10

PATENT APPLICATION
Attorney Docket No. 8616.CIP3

DECLARATION, POWER OF ATTORNEY, AND PETITION

We, **Carl T. Wittwer and David R. Hillyard,** declare: that we are citizens of the United States of America; that our residence and post office addresses are shown below adjacent to our names; that we verily believe we are the original, first, and sole inventors of the subject matter of the invention entitled **METHOD FOR RAPID THERMAL CYCLING OF BIOLOGICAL SAMPLES**, for which a patent is sought and which is described and claimed in the specification filed in the United States Patent and Trademark Office on October 2, 1995 as Serial No. 08/537,612. (We hereby authorize the attorneys of Thorpe, North & Western to insert said serial number when known); that we have reviewed and understand the contents of the above-identified specification, including the claims; and that we acknowledge the duty to disclose information which is material to the examination of this application in accordance with Section 1.56(a) of Title 37 of the Code of Federal Regulations.

We hereby claim the benefit under Section 120 of Title 35 of the United States Code of the earlier filed U.S. patent application serial no. 08/179,969 filed January 10, 1994 entitled Rapid Thermal Cycling Device, U.S. Patent No. 5,455,175 issued on October 3, 1995 which is a continuation-in-part of U.S. patent application 07/815,966 filed January 1, 1992 entitled Rapid Thermal Cycling Device which is a continuation-in-part of U.S. Patent application serial no. 07/534,029 filed June 4, 1990 entitled Automated Polymerase Chain Reaction Device; and, insofar as the subject

matter of each of the claims of these applications are not disclosed in the earlier filed pending applications in the manner provided by the first paragraph of Section 112 of Title 35 of the United States code, we acknowledge the duty to disclose material information, as defined in Section 1.56(a) of Title 37 of the Code of Federal Regulations, which occurred between the filing date of the earlier filed applications and the filing date of this application.

We declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issuing thereon.

We hereby appoint as our attorneys CALVIN E. THORPE, Registration No. 24928, VAUGHN W. NORTH, Registration No. 27930, M. WAYNE WESTERN, Registration No. 22788, GRANT R. CLAYTON, Registration No. 32462, ALAN J. HOWARTH, Registration No. 36,553, KARL R. CANNON, Registration No. 36,468, V. ROLAND SMITH, Registration No. 37,727, SALLY J. BROWN, Registration No. 37,788, and DAVID O'BRYANT, Registration No. 39,793, all of the law firm of THORPE, NORTH & WESTERN located at 9035 South 700 East, Suite 200, Sandy, Utah 84070, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

All correspondence and telephonic communications should be directed to:

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Attorney Docket No. 8616.CIP3

Wherefore, we pray that Letters Patent be granted to us for the invention or discovery described and claimed in the specification and claims, declaration, power of attorney, and this petition.

Signed at Salt Lake City, Utah, this 18th day of October, 1995.

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